Soil organic matter dynamics in a temperate forest influenced by extreme weather events

Dissertation

zur Erlangung des Grades

Doktor der Naturwissenschaften

(Dr. rer. nat.)

an der

Fakultät Biologie/Chemie/Geowissenschaften

der Universität Bayreuth

vorgelegt von

Andrea Schmitt, Diplom-Geoökologin

geb. am 15.11.1969 in Bad Neustadt

Erstgutachter: Prof. Dr. Bruno Glaser

Bayreuth, Juni 2011

Die vorliegende Arbeit wurde in der Zeit von April 2005 bis November 2010 am Lehrstuhl Bodenphysik der Universität Bayreuth unter der Betreuung von Herrn Prof. Dr. Bruno Glaser angefertigt.

Vollständiger Abdruck der von der Fakultät Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades Doktor der Naturwissenschaften (Dr. rer. nat.)

Amtierender Dekan:	Prof. Dr. Stephan Clemens
Tag der Einreichung des Dissertation:	17. November 2010
Tag des wissenschaftlichen Kolloquiums:	22. März 2011

Prüfungsausschuß:

Prof. Dr. Bruno Glaser	(Erstgutachter)
Prof. Dr. Yakov Kuzyakov	(Zweitgutachter)
Prof. Dr. Bernd Huwe	(Vorsitzender)
Prof. Dr. Gerhard Gebauer	
Dr. Werner Borken	

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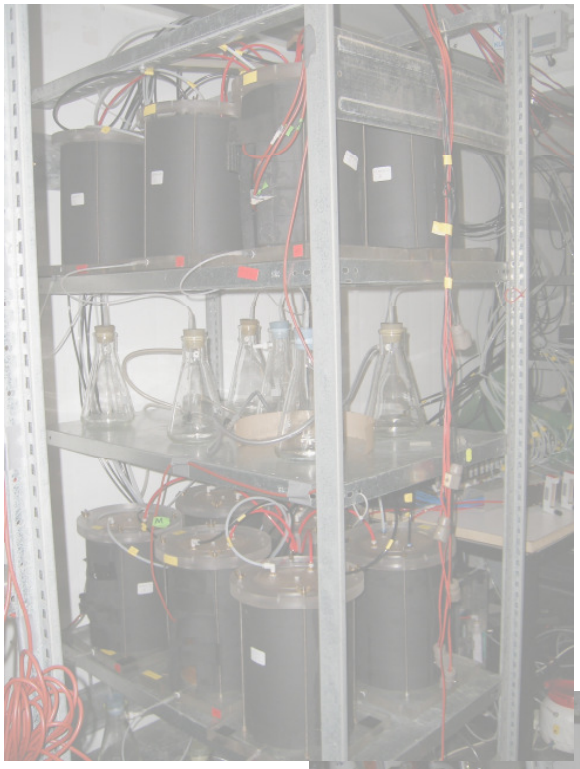

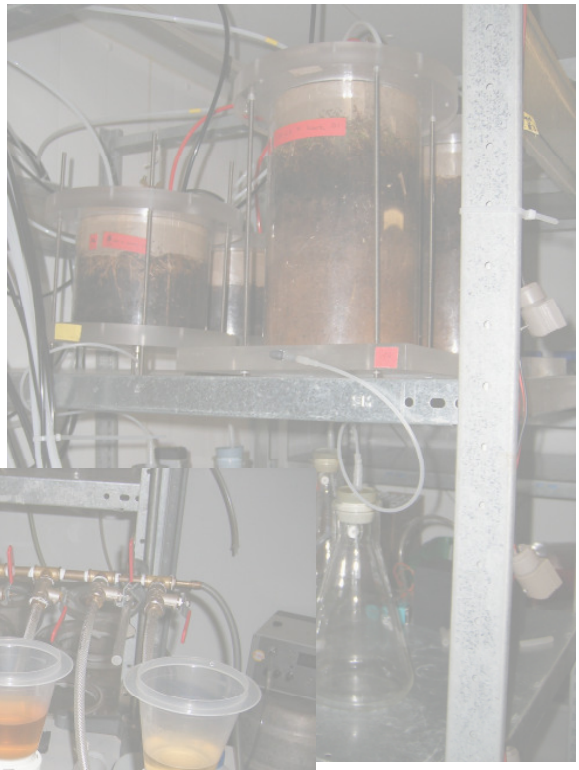

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List of Abbreviations

3 OM	3-O-Methylglucose
^{12}C	stable carbon atom with atomic mass 12
^{13}C	stable carbon atom with atomic mass 13
^{14}C	stable carbon atom with atomic mass 14
a.s.l.	above see level
A	mineral topsoil horizon
B	mineral subsoil horizon
BF_3	Boron Trifluoride in MeOH
BS	Base Saturation
BSTFA	N,O-Bis-(tri-methylSilyl)TriFluoroAcetamide
C	Carbon
C	Cinamyl phenol units
CEC_{eff}	effective Cation-Exchange Capacity
CF-IRMS	Continuous-Flow Isotope Ratio Mass Spectrometry
CSIA	Compound-Specific Isotope Analysis
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
FAME	Fatty Acid Methyl Esters
FID	Flame Ionization Detector
GC	Gas Chromatography
GC-C-IRMS	Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometer
HCl	Hydrochloric acid
IPCC	International Panel of Climate Change
KOH	potassium hydroxid

M	mineral horizon
MBA	Methyl Boronic Acid
MeOH	methanol
NaCl	sodium chloride
NaOH	sodium hydroxid
O	Organic layer
O + M	O layer + mineral horizon
OM	Organic Matter
PCA	Principal-Component-Analysis
PLFA	Phospholipid Fatty Acids
R^2	correlation coefficient
S	Syringyl phenol units
SE	standard error of mean
SM	Snow removal
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TE	Throughfall Exclusion
TFA	trifluoracetic acid
TOC	Total Organic Carbon
V	Vanillin phenol units
VSC	sum of V + S + C
y	year
$\delta^{13}C$	natural abundance of ^{13}C
$\delta^{14}C$	natural abundance of ^{14}C
$(Ac/Ad)_V$	acid-to-aldehyde ratios of vanillin

Summary

Climate models predict an increase in surface temperature and a change in intensity and kind of precipitation in the future for Europe depending on the region. Therefore, under temperate climate, frequency of extreme weather events such as intensive freezing or frequent thawing periods during winter respectively long drying period followed by heavy raining events in the summer might increase with effects on C cycling and soil organic matter (SOM) quality and dynamics. We investigated the influence of extreme weather events (frost / drought) on the quality and quantity of SOM in a Haplic Podzol under a 140 years old Norway spruce forest in the Fichtelgebirge mountains (Bavaria, German) within two laboratory and two field studies. In one laboratory study, we investigated the effect of frost intensity and repeated freeze/thaw cycles. Undisturbed soil columns comprising organic layer and top mineral soil were treated as followed: Control (+5 °C), frost at –3 °C, –8 °C and –13 °C. After a two-week freezing period, frozen soils were thawed at +5 °C and irrigated with 80 mm water at a rate of 4 mm per day. After the third cycle, SOM pools of the treatments were compared with those of non-dried control columns. Under field conditions from late December 2005 until middle of February 2006 we removed the natural snow cover during winter on three replicate plots. Hence we induced soil frost to 15 cm depth (in a depth of 5 cm below surface up to -5°C) from January to April 2006, while the snow-covered control plots never reached temperatures below 0 °C. In the second laboratory experiment after air-drying for five weeks, undisturbed soil columns were re-wetted at different intensities (8, 20 and 50 mm per day) and time intervals, so that all treatments received the same amount of water per cycle (100 mm). After the third cycle, SOM pools of the treatments were compared with those of non-dried control columns. Under field conditions, a throughfall exclusion (TE) experiment was conducted in the summers 2006 and 2007 using a roof installation followed by re-wetting compared to non-manipulated control plots. On 18th January

2007, the heavy low pressure system Kyrill caused large damages at our control plots whereas the TE sites were less influenced. Therefore, for this study, only data were used from the control plots before Kyrill and from the soil structure undisturbed TE plots. SOM quantity and quality was followed by biomarker analysis: lignin, neutral sugars and phospholipid fatty acids (PLFA) as measure for microbial biomass.

Amounts of lignin contents were not significantly affected by repeated freeze/thaw cycles. However, intensive frost slightly enhanced lignin mobilization in the O layer and the translocation into the B horizon. While soil frost did not influence lignin concentrations, the decomposition rate of vanillyl monomers $(Ac/Ad)_V$ decreased at the end of the frost period, these results confirm reduced mineralisation under frost. In contrast, lignin phenols were not systematically affected by the drying/rewetting-experiment and the moisture regime.

The sum of PLFA (soil microbial biomass) was not affected by the frost respectively drying event, suggesting that most soil microorganisms were well adapted or recovered more quickly than the accumulation of microbial residues such as microbial sugars directly after the experiment. However, PLFA patterns indicate that fungi are more susceptible to soil frost than bacteria. The ratio of fungi to bacteria were generally not altered through drying, however, at least in the L horizon, warmer and drier weather led to a dominance of fungi while a cooler and moister regime favoured bacteria. Increasing water stress was indicated by a higher PLFA (cy17:0+cy19:0)/ (16:1ω7c+18:1ω7c) ratio suggesting that the microbes suffered from water stress in the organic layer and uppermost mineral soil. While soil microbial biomass was not affected by the moisture regime, the structure of soil microbial community changed. Gram-positive bacteria and actinomycetes were reduced whereas gram-negative bacteria, fungi and protozoa were stimulated by the reduced moisture regime. In the subsequent summer after the freezing experience, soil microbial biomass was significantly higher at

the snow-removal plots (SM) compared to the control despite lower CO_2 respiration and increasing water stress indicator. These results suggest that soil microbial respiration and therefore the activity was not closely related to soil microbial biomass but more strongly controlled by substrate availability and quality.

Both freezing/thawing and drying/re-wetting reduced the amount of microbial sugars due to reduced mineralisation. However, also the hydrolysable plant sugars decreased in all soil horizons. We postulated that the only possible explanation for the disappearance of plant and microbial sugars upon soil freezing or drying are chemical alterations of sugar molecules leading to SOM stabilization, also known as SOM aging. Further studies are required to quantify the effect of temperature or moisture regime to the observed changes in soil sugar concentrations.

Zusammenfassung

Für die Zukunft, prognostizieren Klimamodelle für Europa, dass die globale Temperatur ansteigt, abhängig von der Region, und eine Veränderung in der Intensität und Art der Niederschläge zu beobachten sein wird. Aufgrund der Erwärmung fallen im Winter die Niederschläge eher als Regen, statt als Schnee. Dadurch können trotzdem auftretende Fröste aufgrund der fehlenden schützenden Schneedecke tiefer in den Boden eindringen und länger anhalten, oft unterbrochen durch häufigere Auftauphasen. Im Sommer dagegen ändert sich die Summe der Niederschlagsmenge nicht, jedoch ihre Verteilung. Lange Trockenphasen werden durch heftige Starkregenereignisse abgelöst, was auch jetzt schon zunehmend zu beobachten ist. Diese Veränderungen des Klimas in Mitteleuropa haben Auswirkungen auf den C-Kreislauf und auf die Dynamik der organischen Bodensubstanz (OBS). Daher war das Ziel dieser Studie, den Einfluss von Extremwetterereignissen anhand von Biomarkern [Lignin, pflanzliche und mikrobielle Zucker und Phopholipidfettsäuren (PLFA)] auf die OBS-Qualität und -Quantität in einen Haplic Podzol unter einem 140 Jahre alten Fichtenbestand im Fichtelgebirge zu untersuchen mittels zweier Labor- und zweier Freilandexperimenten.

In einem Laborexperiment untersuchten wir den Effekt von Frostintensität und wiederholten Gefrier-/Auftauphasen (der Versuch wurde mit den gleichen Bodensäulen 3 mal wiederholt). Ungestörte Bodensäulen gefüllt mit organischer Auflage und Mineralboden wurden folgendermaßen behandelt: Kontrolle (+5 °C), gefroren bei –3 °C, –8 °C und –13 °C. Nach einer zweiwöchigen Gefrierphase, wurden die gefrorenen Bodensäulen bei +5 °C aufgetaut und anschließend mit einer Rate von 4 mm pro Tag (insgesamt 80 mm) beregnet. Im Freiland wurde von Ende Dezember 2005 bis Mitte Februar 2006 auf drei Wiederholungen die natürliche Schneedecke entfernt. Dadurch induzierten wir bis zu einer Tiefe von 15 cm Bodenfrost (in einer Tiefe von 5 cm

erreichte die Bodentemperatur bis zu -5 °C) von Januar bis April 2006, während bei der schneegeschützten Kontrolle die Bodentemperatur nie unter 0 °C lag.

Im anderen Laborexperiment wurde die luftgetrockneten ungestörten Bodensäulen wiederberegnet mit unterschiedlichen Intensitäten (8, 20 and 50 mm pro Tag) und Zeitintervallen, so dass alle Behandlungen die gleiche Regenmenge (100 mm) pro Durchgang erhielten. Auch hier wurde das Experiment mit den gleichen Bodensäulen 3 mal durchgeführt, während die Kontrolle nie getrocknet wurde. Unter Feldbedingungen wurde sowohl im Sommer 2006 als auch 2007 ein Austrocknungsexperiment mit Hilfe von Dachinstallationen erstellt. Nur 2006 wurden die fehlenden 70 mm Niederschlag am Versuchende mit einer Sprinkleranlage innerhalb von 2 Tagen wieder zugeführt. Am 18. Januar 2007, verursachte das Sturmtief Kyrill große Schäden auf unseren Kontrollplots, während die Austrocknungsflächen (TE -plots) kaum beeinflusst wurden. Somit wurde für die Auswertung dieser Studie entschieden, nur die Daten der der Kontrolle vor dem Kyrill-Ereignis und den ungestörten TE -plots zu verwenden.

Die Summe des Ligningehalts wurde durch wiederholte Gefrier-Auftau-Ereignisse nicht signifikant beeinflusst. Allerdings führten intensive Gefriertemperaturen zu einer erhöhten Ligninmobilität von der organischen Auflage und zu einem Transport in den B Horizont. Während der Bodenfrost die Ligninkonzentration kaum beeinflusst hat, nahm das Säure-Aldehyd-Verhältnis von Vanillin $(Ac/Ad)_V$ am Ende der Frostperiode ab, dieses Ergebnis bestätigte die reduzierte Mineralisation unter Frost. Im Gegensatz dazu wurde Lignin durch Austrocknung/Wiederbefeuchtung und dem daraus resultierenden Feuchtegehalt nicht systematisch beeinflusst.

Die mikrobielle Biomasse wurde durch Frost- bzw. Austrocknung nicht beeinflusst, was die Vermutung nahelegt, dass die Summe der Bodenmikroorganismen

gut an den Klimawandel im Fichtelgebirge angepasst ist. Allerdings konnte eine Verschiebung der Mikroorganismenpopulation beobachtet werden. So wiesen PLFA-Muster darauf hin, dass Pilze mehr unter Bodenfrost leiden als Bakterien. Das Verhältnis von Pilzen zu Bakterien änderte sich aufgrund der Trockenheit im Allgemeinen nicht, jedoch, zumindest im L Horizont, führte wärmeres und trockeneres Wetter zu einer Dominanz der Pilze, während kühleres und feuchteres Regime Bakterien förderte. Der ansteigende Wasserstressindikator [PLFA (cy17:0 + cy19:0) / (16:1w7c+18:1w7c; abgekürzt als cy/pre)] legt die Vermutung nahe, dass die Mikroben in der organischen Auflage und oberen Mineralhorizont unter Wasserstress litten. Mit abnehmendem Wassergehalt waren Gram-positive Bakterien und Actinomyceten reduziert, dagegen wurden Gram-negative Bakterien, Pilze und Protozoa stimuliert. Im darauf folgenden Sommer nach dem Gefrierereignis fand man auf den Gefrierflächen eine signifikant höhere mikrobielle Biomasse als auf der Kontrolle, trotz niedrigerer CO_2 Atmung und ansteigendem Wasserstress. Dieses Ergebnis gibt einen Hinweis darauf, dass die Bodenatmung und daraus resultierenden Aktivität der Mikroorganismen nicht in enger Beziehung zur mikrobiellen Biomasse steht, sondern von der Substratverfügbarkeit und Qualität beeinflusst wird.

Sowohl Gefrieren/Auftauen als auch Austrocknung/Wiederberegnung reduziert die Summe der mikrobiellen Zucker aufgrund reduzierter Mineralisation. Jedoch auch pflanzliche Zucker haben in allen Bodenhorizonten abgenommen. Die einzige mögliche Erklärung für das Verschwinden der pflanzlichen und mikrobiellen Zucker aufgrund von Bodenfrost oder Trockenheit ist die chemische Umwandlung der Zuckermoleküle, was zu einer SOM Stabilisierung („Aging“) führt.

1 Introduction

1.1 Climate change

Climate models predict an increase in global surface temperature and a change in the intensity of precipitation in this century (IPCC, 2001; 2007). On the one side in the summer during the last decades, extended droughts followed by heavy rainfall were already observed in Europe (Foken, 2004). On the other side with the tendency of increasing global surface temperature, intensity and frequency of soil frost may change in the future. A lack of snow cover or late snowfall in winter results in soil freezing that is deeper and of longer duration than when early snowfall occurs (Fitzhugh et al., 2001). Alternatively, more frequent thawing periods during winter may occur.

Therefore, climate change might influence amount, quality and turnover of SOM. Important SOM constituents are primary litter components such as cellulose, hemicellulose (sugars) and lignin as well as secondary microbial products such as deoxy sugars and phospholipids (Guggenberger et al., 1995; Amelung et al., 1997; 1999; Guggenberger et al., 1999; Koegel-Knabner, 2000; Gleixner et al., 2002).

1.2 Lignin phenols

Lignin is a main component in forest litter and represents a major input of organic matter into forest soils (Ziegler et al. 1986). Lignin compounds are phenolic polymers consisting of vanillin (V), syringyl (S), and cinamyl (C) moieties found in the cell walls of all vascular plants (Hedges and Ertel, 1982). The sum of V+S+C (VSC) after alkaline CuO oxidation was adopted as an indicator of the amount of intact lignin moieties (Ziegler et al., 1986).

The intermediate or slow SOM pool comprises structural plant residues with a MRT of 25 – 50 years and due to specific structural properties, lignin phenols are recalcitrant against microbial decomposition and contribute mainly to the long-term C -pool of forest floors

(Haider, 1996), with a mean residence time of 1,000 – 1,500 years (Cochran et al. 2007). But this doctrine is also disputed. Other authors (Kiem and Kogel-Knabner, 2003; Dignac et al., 2005) detected faster apparent turnover of lignin, for example 5 – 26 years in grassland and 9 – 38 years in arable soils (Heim and Schmidt, 2007), and lignin did not accumulate within the refractory C pool. The two-reservoir-model of Rasse et al. (2006) indicated chemical recalcitrance alone is not sufficient to explain VSC -lignin turnover in soils and the most relevant mechanisms appears to be the transfer of VSC -lignin molecules and fragments from decomposing plant tissue to soil protected fractions. However, all of these authors worked with samples of soil types of European agro ecosystems without organic layer and cultivated plants like maize. Hence, it is difficult to estimate how far these results could be transferred to a forest soil with a spruce stock. So we classified lignin nevertheless as stabile carbon pool due to specific structural properties, like Haider (1996) and Cochran (2007).

The gymnosperm lignin consist to 80 % coniferyl alcohol-derived units (vanillyl phenols) (Ziegler et al., 1986) and the lignin molecule is typically altered during decomposition by white-rot fungi oxidizing aldehyde units and produce acid units (Koegel, 1986). Therefore, the state of degradation can be recognized by the ratio of the oxidized derivatives versus the corresponding aldehyde $(Ac/Ad)_V$ of the vanillyl monomers (Koegel-Knabner, 2000; Otto and Simpson, 2006).

1.3 Plant and microbial sugars

Non-cellulosic sugars are important SOM constituents being most abundant in root exudates and non-structural plant constituents (Derrien et al., 2004). While the pentoses arabinose and xylose are mainly plant-derived, hexoses and deoxy sugars such as fucose and rhamnose are of microbial origin (Amelung et al., 1996). Advanced biodegradation of SOM shifts the composition of neutral sugars from pentoses to deoxy sugars indicating consumption of plant-derived organic matter and production of microbial compounds. The ratio of arabinose +

xylose to fucose + rhamnose is a tool to trace effects of environmental changes on SOM degradation (Oades, 1989; Guggenberger and Zech, 1994; Amelung et al., 1999; Glaser et al., 2000).

1.4 Phospho lipid fatty acids (PLFA)

Soil phospho lipid fatty acid (PLFA) content is a measure for soil microbial biomass (Frostegard et al., 1991) correlating with the chloroform fumigation extraction method in forest floors (Leckie et al., 2004), whereas individual PLFA are characteristic for different microbial groups (Frostegard et al., 1993; Cavigelli et al., 1995; Zelles, 1999). As PLFA are subject to rapid decomposition after death of the microorganisms, they characterize the current microbial community (White et al., 1979; Zelles et al., 1992; Leckie et al., 2004).

Terminal branched satured PLFA (a15:0, i15:0, i16:0, i17:0, a17:0) are considered as makers for gram-positive bacteria and mid-chain branched saturated PLFA (10Me16:0, 10Me17:0, 10Me18:0) were associated with actinomycetes. Typical for gram-negative bacteria are monounsaturated fatty acids (16:1ω7c, 18:1ω7c) and cyclopropyl saturated PLFA (cy17:0, cy19:0). Short or odd-chain saturated PLFA (14:0, 15:0, 16:0, 17:0, and 18:0) were considered non-specific bacterial makers and are present in all microbial organisms. Typical markers for fungi are PLFA 18:2ω6,9, 18:1ω9c and 16:1ω5c (Stahl and Klug, 1996; Zelles, 1999; Myers et al., 2001; Ruess et al., 2002; Deforest et al., 2004; Waldrop et al., 2004; Mcmahon et al., 2005).

1.5 Objectives

Under the impact of extended summer droughts and rewetting respectively freezing and thawing, soils undergo complex changes of soil structure (aggregation), soil organic matter and microflora (Denef et al., 2001). These events produce a significant stress on soil

microbial communities and induce significant changes in microbial C dynamics (Fierer and Schimel, 2002). Thus, a shift in the amount, intensity and frequency of precipitation could change the C storage and their compounds in temperate forest ecosystems (Borken and Matzner, 2009). The C turnover affected by frost respectively by drying was well examined until now, but the attention was usually more directed towards how the stress-situation affected dissolved organic carbon (DOC) (Christ and David, 1996; Lamersdorf et al., 1998; Borken et al., 1999; Tipping et al., 1999; Hentschel et al., 2007; Agren et al., 2008; Hentschel et al., 2008; Hentschel et al., 2009, Schulze et al., 2010), respiration (CO_2) (Clein and Schimel, 1994; Brooks et al., 1997; Borken et al., 1999; Franzluebbers et al., 2000; Fierer and Schimel, 2002; 2003; Brooks et al., 2005; Wu and Brookes, 2005; Larsen et al., 2007; Goldberg et al., 2008; Muhr et al., 2008; Muhr and Borken, 2009; Muhr et al., 2009) or total soil organic carbon (TOC, SOC) (Degens and Sparling, 1995; Pulleman and Tietema, 1999; Fitzhugh et al., 2001; Groffman et al., 2001; Neilson et al., 2001; Haney et al., 2004; Mikha et al., 2005; Austnes et al., 2008; Jansson et al., 2008; Michaelson et al., 2008). However, our knowledge about the response of microbial communities to freezing and thawing respectively drying and rewetting is sparse. Biomarker profiles provide information about changes in the community of soil microorganisms or in the use of substrates following such stress situations. Hence, the focus of this work was on the characterization of different SOC pools by means of the biomarkers lignin (stable pool), plant and microbial derived sugars (labile pool) and phospho lipid fatty acids (PLFA) as measure for the microbial biomass and its community structure.

We hypothesize i) a shift of the microbial community as indicated by an increasing ratio of fungal PLFA to bacterial PLFA and enhanced physiological/nutritional stress as indicated by an increase of the cy/pre ratio. Further, we hypothesize ii) a reduction of microbial sugars, relative accumulation of plant-derived sugars and lignin phenols because of decreasing microbial activity in soils during freezing respectively drought and iii) that these

effects will be strongest in the mineral soil since organic horizons are better adapted to extreme changes of temperature respectively of moisture.

2 Experimental design

2.1 Study area (Study 1 - 4)

The experimental site (Coulissenhieb II) is a 140-years-old Norway spruce (*Picea abies (Karst.) L.*) stand located in the Fichtelgebirge mountains (Northern Bavaria, Germany) at about 770 m above sea level classified as Haplic Podzol (see Table I for basic soil properties). The annual temperature in the Fichtelgebirge area is +5.3 °C and the annual precipitation is 1,160 mm (Gerstberger et al., 2004). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

Table I: Chemical properties of nine soil profiles in the Norway spruce stand at the Fichtelgebirge (n=9). (CEC_{eff} = effective-cation-exchange capacity; BS = base saturation).

	depth	pH		C	N	C/N	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Al^{3+}	H^{+}	CEC_{eff}	BS
	cm	H_2O	$CaCl_2$	–[%] ——			—————— [$mmol_c\ kg^{-1}$] ——————							%
O_a	5	4.0	3.3	18.0	1.0	17.7	94.4	7.6	1.4	3.4	74.1	8.0	197	54
Ah	-5	4.3	3.4	7.4	0.4	19.2	60.9	3.6	1.0	1.9	66.9	7.9	145	47
Bh	-12	4.3	3.4	5.5	0.3	19.7	70.9	2.3	1.1	1.3	97.6	4.0	185	41
Bs	-18	4.6	3.7	3.4	0.2	20.5	30.1	1.1	0.9	1.0	87.8	1.3	124	27
Bw	-55	4.6	4.1	1.3	0.1	12.3	3.7	0.2	1.1	1.0	41.3	0.4	48	12
Bw/C	<-55	4.5	4.0	0.4	0.0	8.3	2.7	0.2	3.0	1.1	35.3	0.2	43	16

Two different types of soil columns were sampled using plexiglass cylinders (diameter 17.1 cm). Cylinders with a height of 15 cm were used for sampling organic layers (hereafter O columns), whereas cylinders with a height of 30 cm were used for sampling Oi, Oe, Oa, Ea, Bhs and Bsh horizons (hereafter organic + mineral (O+M) columns).

2.2 Laboratory experiment: freeze-thawing cycle (Study 1)

Four different treatments with four replicates for both O and O+M columns were established to study the effect of repeated freezing and thawing: Control (+5°C) and frost at –3°C, –8°C

and –13°C (Table II). After two weeks of freezing, the columns were thawed in a +5 °C climate room and irrigated during 20 days at 4 mm daily with an artificial solution corresponding to natural precipitation (Matzner et al., 2004). The freezing /thawing/irrigation cycle was repeated three times with the same columns under the same conditions.

Table II: Experimental design for the laboratory experiment: freeze-thawing cycle.

Parameter	**Control**	**I**	**II**	**III**
Freezing temperatur (14 days) [°C]	+5	-3	-8	-13
Thawing temperatur [°C]	+5	+5	+5	+5
Thawing period [d]	7	7	7	7
Raining intensity [mm d^{-1}]	4	4	4	4
Raining amount [mm]	80	80	80	80
Raining period [d]	20	20	20	20

Total amount of precipitation approx. 1800 ml

2.3 Laboratory experiment: repeated drying-rewetting (Study 2)

Four different treatments with four replicates for both O and O+M columns were established to study the effect of repeated drying and re-wetting: Control (4 mm precipitation) and three drying and re-wetting treatments (8, 20 and 50 mm precipitation; Figure I). All drying and re-wetting treatments were air-dried to the permanent wilting point (approximately -1,500 kPa) at 20 °C for 5 – 6 weeks and re-wetted at 15 °C. All three treatments received 100 mm solution during the intensive re-wetting period and after this period; all treatments were uniformly irrigated at 4 mm for 14 days. The controls were permanently irrigated at 4 mm per day during the intensive rewetting and post-re-wetting periods. All control and drying and re-wetting treatments received the same artificial solution corresponding to natural throughfall at the experimental site (Matzner et al., 2004). The same columns were subjected to three drying and re-wetting cycles under the same conditions.

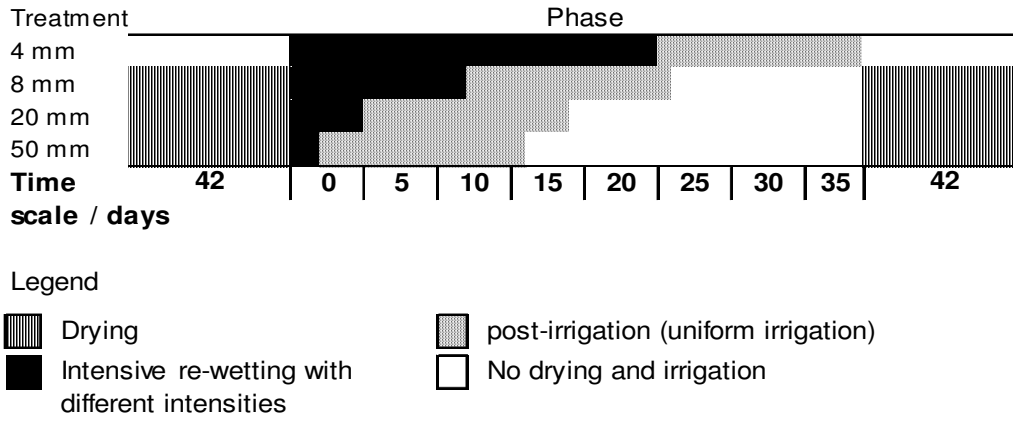

Figure I: Schematic schedule of one drying and re-wetting cycle, consisting of i) drying, ii) intensive re-wetting period, during which re-wetting intensity was varying for the different treatments and iii) a post-irrigation period with uniform irrigation in all treatments. The control (4 mm of solution) was not subjected to drying and was irrigated with 4 mm d^{-1} during the intensive re-wetting and post-irrigation periods. All treatments received the same amount of water (156 mm) during each drying and re-wetting cycle.

2.4 Field experiment: snow removal (Study 3)

Six plots, each of them of 20 x 20 m, were established during the summer 2005 in the Fichtelgebirge, 3 for the control and 3 as snow removal areas. From late December 2005, before the first frost, the fresh-fallen snow was removed by hand from the snow removal plots (SM). After the end of the manipulation, the removed snow was not returned, therefore on the snow removal plots throughfall of 147 mm was missing. The end of the snow removal period was set for the early February 2006. During winter 2005/06 plenty of snow and frost continued until end of March and therefore a snow cover could accumulate and insulated the soil frost until April 2006. The control plots were undisturbed during the experiment and the thickness of the total snow cover accounted for 50 - 80 cm. For the analyses of the biomarkers two cores (20 cm long and 7 cm diameter) of each plot (SM -plots and controls) were taken for a mixed sample before (2005) and after (0406) the experiment.

For the winter 2006/07 a repetition of the experiment could not be carried out for two reasons. First, the winter was to warm with a lack of snow and frost, and second there was a heavy storm called “Kyrill” in January 2007, which disturbed the installation of the plots.

2.5 Field experiment: throughfall exclusion (Study 4)

Six plots were established, 3 as control (C) and 3 as throughfall exclusion (TE) plots. During summer 2005, wooden roof structures of about 400 m² (20 x 20 m) and 3 m height were constructed at the TE sites. Between 22nd June and 8th August 2006, the roof construction was covered with transparent plastic panels (Owolux 76/18, 1140 x 2500 mm) to simulate summer dryness as the precipitation water was kept out. Through the roof construction, about 70 mm throughfall were excluded which were re-applied after the end of the experiment within 2 days. After re-wetting, the plastic panels were removed. Between 3rd July and 13th August 2007 the drying experiment was repeated. However, this time at the end of the manipulation, the TE plots were not re-wetted. For the biomarker analyses two cores (20 cm long and 7 cm diameter) of each plot (TE –plots and controls) were taken for a mixed sample before (2005 respectively 0607) and after (0806 respectively 0807) the experiment.

On 18th January 2007, the heavy low pressure system Kyrill moved over our experimental site with almost no destruction at the TE plots, while at the control plots, some trees were pulled out including the roots, thus destroying the soil structure. Although trees were also removed subsequently at the TE plots for homogenization purposes, the interpretation of the results of the year 2007 and the comparability with the control sites have thus to be treated with caution.

3 Methods

3.1 Sampling

Soil solution (DOM) in each case from the last laboratory cycles were filtered through 0.45 µm cellulose-acetate-membrane filters (Schleicher & Schüll, 37586 Dassel, Germany). For biomarker analysis, mixed solutions were prepared combining 60 % of total percolate amount from individual sampling dates of the last drying-rewetting cycle. Composite DOM samples

were freeze-dried prior to analysis.

All soil samples were separated into organic layer (L and O horizons) and mineral soil (A and B horizons). The organic horizons were cut into small pieces, mixed and the mineral horizons were sieved < 2 mm. For PLFA analysis, an aliquot of fresh samples was kept frozen < –20 °C. For sugar and lignin analysis, the samples were dried at 40 °C and ground. For every horizon the gravimetric water contents (dried at 105 °C) was determined. For the laboratory experiment, the concentrations of total organic C (TOC) was analyzed using a Vario EL elemental analyzer (Elementar, Hanau, Germany) and for the field experiment a EA-IRMS (MS: Finnigen Delta S, EA: fisons EA1108).

3.2 Lignin analysis

The samples with an equivalent of dry soil corresponding to 25 mg TOC (10 mg C of freeze-dried material) were oxidized with a modified alkaline CuO to release lignin-derived phenols (modified after Hedges and Ertel, 1982). For quantification, derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID).

3.3 Sugar analysis

Samples for plant and microbial derived sugar extraction with an equivalent of dry soil corresponding to 8 mg TOC (6 mg C of freeze-dried material) was carried out according to a modified Amelung et al. (1996) method. The derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID).

3.4 Phospholipid fatty acids (PLFA) analysis

PLFA extraction from 5 g fresh soil and freeze-dried DOM (corresponding to 10 mg TOC) was carried out according to a modified Frostegard et al. (1991) method. The Fatty acid

methyl esters (FAME) were prepared from free PLFA using a strong acid methylation. The derivates were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID).

3.5 Statistical analysis

Statistical analyses were carried out using STATISTICA 5.0. Differences between different treatments were evaluated using a one-way ANOVA followed by the Tuckey-Honest post-hoc test. The coefficients of correlation were computed with Excel 2003.

4 Results and Discussion

4.1 Influence of frost (Studies 1 and 3)

4.1.1 Lignin phenols

Both in the laboratory and the field experiments, lignin was not affected through the induced freezing events. However, intensive frost slightly enhanced lignin mobilization in the O layer and the translocation into the B horizon in the laboratory experiment (Figure IIa).

Soil freezing may have induced physical disruption of soil aggregates and a release of otherwise physically protected particulate SOM (Bullock, et al. 1988) and microbial products (Skogland et al., 1988). Thus, intensive frost (> -8 °C) led to physical-chemical changes of SOM and to slightly enhanced lignin solubility in the litter layer as indicated by higher lignin concentrations in DOM leached through the O layer (Figure IIb). Also Kawahigashi et al. (2004) observed higher DOC concentrations and contribution of hydrophobic compounds such as lignin with increasing permafrost intensity (-0.1 to -2.5 °C) in water samples along the Yenisei River between 69°30'N and 65°49'N latitude in Siberia which is in accordance with our results.

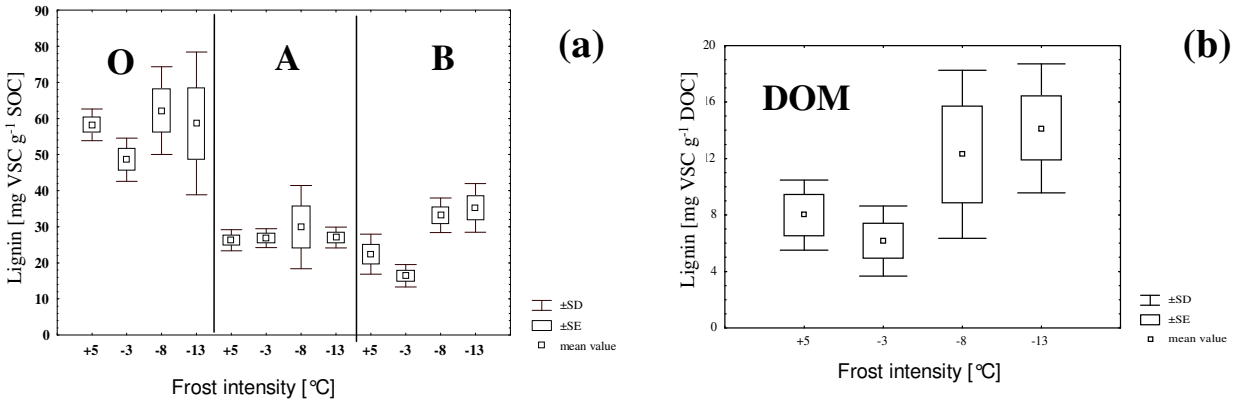

Figure II: Content of lignin phenols (sum of VSC) (a) in contribution to SOC in the O, A and B horizons and (b) in soil solution from the O horizons after the third freezing and thawing cycle of the control (+ 5°C), -3 °C, -8 °C and -13 °C treatments.

4.1.2 Plant and microbial sugars

Plant and microbial sugar concentrations decreased with increasing frost intensity. Decreasing microbial sugars with increasing frost intensity indicate reduced microbial activity in the same direction corroborating decreasing mineralization rates (CO_2 respiration) with increasing frost intensity in the 3rd cycle of the laboratory experiment (Goldberg et al., 2008) respectively the field experiment (Muhr et al., 2009). The authors reported that soil respiration fluxes from the snow removal plots (around 1 mmol CO_2 m^{-2} h^{-1}) were almost constantly less than CO_2 fluxes of the control plots (1 – 3 mmol CO_2 m^{-2} h^{-1}) during the experiment.

If microbial activity is reduced under frost, plant sugar concentrations should be similar to the controls, but this was not the case. Instead, also plant sugars decreased with increasing frost intensity. Increased mineralization and mobilization and translocation of the sugars with DOM can be ruled out as explaining this phenomenon. On the one hand the CO_2 emissions decreased and on the other hand an annual throughfall of 147 mm was missing at the snow removal plots. Thus, physical changes of soil structure and / or organo-mineral stabilization of sugars might be responsible for the changing amounts of extractable sugars. For instance, it is well known that freeze/thaw events affect soil structure (Tisdall and Oades, 1982), which influence C availability, aeration and water and nutrient movements, all of which have important effects on quantity and quality of SOM.

Additionally, it cannot be completely ruled out that the analytical method itself is responsible for the observed changes in sugar concentrations as direct contact of extraction solution with SOM is necessary for carbohydrate monomer release. Physical-chemical changes of soil structure can theoretically change also sugar extractability from soils although the applied method was also able to quantify organo-mineral stabilization of sugars in soil (Spielvogel et al., 2007). Nevertheless, if lower sugar extractability would be responsible for our findings, the ecological consequences would be the same as for a decrease of sugar concentrations due to soil structure changes, namely, transformation of a labile into a stable SOM pool.

A further possible explanation is enhanced plant and microbial sugar production in the control treatment compared to the more severe frost treatments e.g. by exo-enzymes being responsible for the observed differences in sugar concentrations. However, as sugar concentrations of an air-dried soil were higher than of the same soil (not air-dried) kept in a refrigerator (+4 to +8 °C, 4 weeks) or a freezer (-18 °C, 4 weeks) sugar production in less frozen soil can be ruled out as explanation for decreasing sugar concentrations with increasing frost intensity (Figure III).

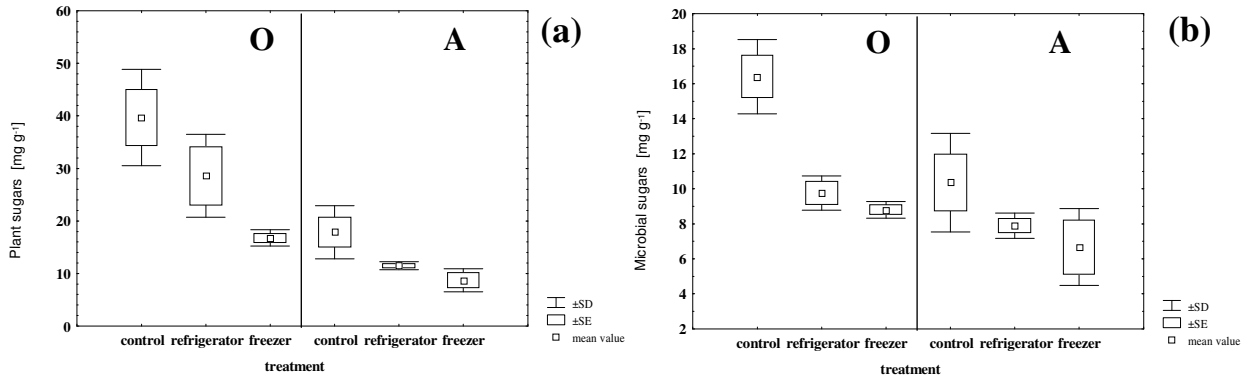

Figure III: (a) Plant and (b) microbial sugar concentrations in organic layer (O) and mineral soil (A) after air-drying, cooling (4 weeks at +4 to +8 °C) and freezing (4 weeks at -18 °C).

Therefore, as both reduced mineralization and physical stabilization can be ruled out, chemical alteration and stabilization is responsible for sugar decrease with increased soil frost

intensity.

4.1.3 Phospho lipid fatty acids (PLFA)

No frost effect was observed with respect to soil microbial biomass both in the laboratory and the field experiments (Figure IVa). Koponen et al. (2006) observed that soil microbial community structure and biomass analysed with PLFA were not affected by freeze/thaw events. The more or less constant PLFA concentrations can be explained by the fact that microbial processes are still active in cold (0 to 5°C) and even frozen soils (Brooks et al., 1998). The critical temperature for active respiration and therefore growth is assumed to be between -7 °C and -5 °C. Lipson et al. (2000) suggested that microbes may adapt to stress they experience regularly, such tolerance has been observed for example in alpine tundra or in Antarctic soils. Also the tendency decreasing cy/pre-ratio at the end of the SM experiment in April 2006, both at control and SM plots (Figure IVc) supported the microbial community did not suffer under moderate freezing stress.

Between April 2006 and November 2006 (over the period of the summer following the snow removal experiment) the sum of the microbial biomass (PLFA) increased significantly ($p < 0.05$) above all in organic and mineral horizons at the SM plots (Figure IVa). Mortality of soil microbial biomass (Jenkinson and Powlson, 1976) or physical disruption of soil aggregates (Vangestel et al., 1992) leads to a marked increase in C mineralization and the survived micro organisms can multiply rapidly, the length of fungal hyphae and bacterial biomass increase (Jager and Bruins, 1974). However, Feng et al. (2007) ascertained the sum of microbial biomass was not closely related to soil microbial respiration but more strongly controlled by substrate availability and quality. Muhr et al. (2009) observed an annual soil respiration flux of 5.1 t C ha^{-1} a^{-1} at the snow removal plots and of 6.2 t C ha^{-1} a^{-1} at the control. The authors reported only 14% of the reduced soil respiration through the snow removal experiment were attributed to the soil frost period itself, whereas 63% of this losses

caused by the summer dryness. Therefore, even if soil microbial biomass pool was not affected, the activity was significantly reduced during the summer dryness. Also the significantly ($p < 0.05$) increasing cy/pre-ratio between April and November 2006 (Figure IVc) suggests a long-term environmental stress (i.e. suffered more from water stress) during the following summer at the SM plots in both horizons.

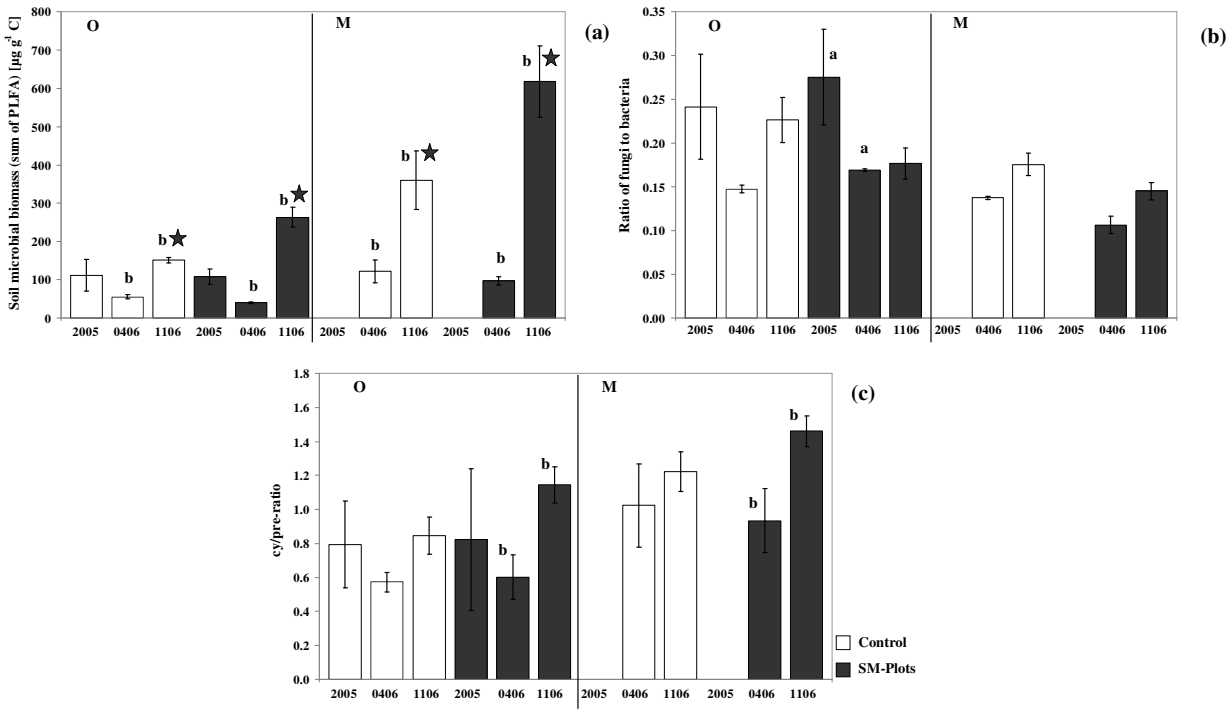

Figure IV: Mean value (± SE) of (a) microbial biomass (sum of PLFA) contribution to TOC [µg g^{-1} C], (b) ratio of fungal to bacterial PLFA and (c) the ratio of the sum of cyclopropyl PLFA to the sum of their monoenoic precursors [(cy17:0+cy19:0)/(16:1ω7c+18:1ω7c; abbreviated as cy/pre)] in the organic layer (O) and mineral horizon (M) before (2005) and after the snow removal (SM) experiment (0406), respectively after the following summer (1106) at the control (white bars) and the SM plots (black bars) (✯ = significant ($p < 0.05$) differences between C and SM plots, a = significant ($p < 0.05$) differences between sample day 2005 and 0406, b = significant ($p < 0.05$) differences between sample day 0406 and 1106)

Even if the sum of soil microbial biomass was not affected trough the frost regime, however, structure of soil microbial community. The frost treatment influenced fungi more negatively than bacteria, a conclusion that is in accordance with literature findings (Figure IVb): Also Feng et al. (2007) and Hoegberg (2006) found decreasing fungal PLFA concentrations upon various disturbances of boreal forest ecosystems. The reduced ratio of fungi to bacteria did not increase again in the organic layer during the following summer after freezing experience, in contrary to control or mineral horizon. Therefore, a long-term effect to

the structure of the microbial community could be observed in the organic horizon.

4.2 Influence of drying (Studies 2 and 4)

4.2.1 Lignin phenols

Both the laboratory and the field experiments demonstrated no significant influence of drought and re-wetting on VSC- lignin. This result was also confirmed by the correlation between VSC- lignin contribution to SOC and the gravimetric water content (L horizon R^2 = 0.26, O horizon R^2 = 0.09, A horizon R^2 = 0.07 and B horizon R^2 = 0.37) respectively the correlation coefficient between $(Ac/Ad)_V$ and the gravimetric water content (L horizon R^2 = 0.06, O horizon R^2 = 0.05, A horizon R^2 = 0.00 and B horizon R^2 = 0.09).

4.2.2 Plant and microbial sugars

In the laboratory experiment, the sum of plant sugars decreased significant ($p < 0.05$) in the organic layer and mineral horizons from the control to the treatments with the most intensives re-wetting. An increased decomposition after rewetting could not be responsible for the decreasing of the plant sugars with the treatments, as Muhr et al. (2008) could not observe that after the rewetting the CO_2 fluxes quickly recovered back to control level. Thus it was improbable, that after the remoistening more plant sugar was converted by the micro organisms. Leaching could be also not responsible for the decreasing plant sugar after rewetting, as the sugars in contribution to DOC did not increase with rewetting intensity in the organic layer.

In the field experiment, the plant sugars in contribution to TOC (Figure Va) significantly correlated positively with the gravimetric water content in the L, O and A horizons (L horizon R^2 = 0.84, $p < 0.01$; O horizon R^2 = 0.65, $p < 0.05$; A horizon R^2 = 0.80, $p < 0.01$ and B horizon R^2 = 0.23). Although Zwiazek (1991), Tan et al. (1992) and Buljovcic

and Engels (2001) found the sugar content in the plant (the starting material) correlated negatively with the moisture regime.

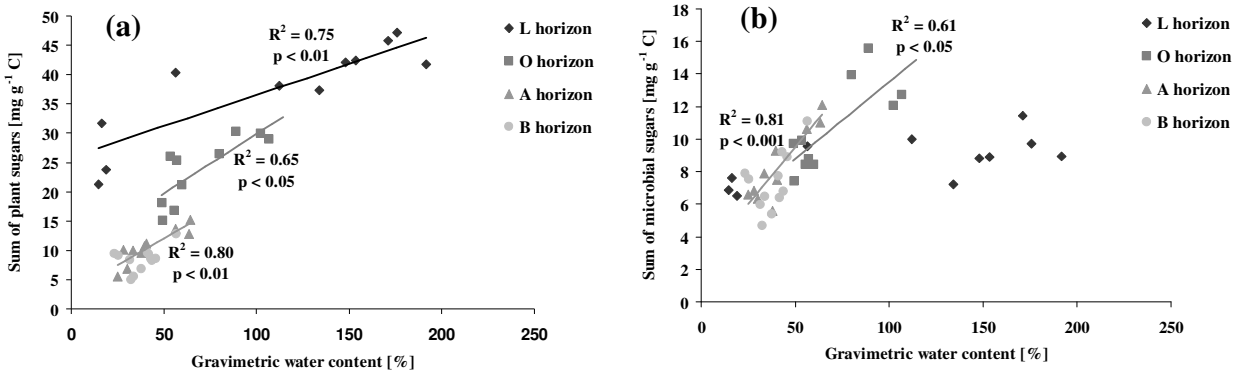

Figure V: Correlation between the gravimetric water content [%] and (a) sum of plant sugars [mg g^{-1} SOC], (b) sum of microbial sugars [mg g^{-1} SOC] without control 0607 and 0807 in the L horizon, organic layer (O), A and B horizon.

Thus, physical changes of soil structure and / or organo-mineral stabilization of sugars might be responsible for the changing amounts of extractable sugars, like the freezing experiments. Under the impact of drying and re-wetting, soils undergo also complex -changes of soil structure (aggregation), soil organic matter and microflora (Denef et al., 2001), which influence C availability, aeration and water and nutrient movements, all of which have important effects on quantity and quality of SOM.

Soil microbial sugars in contribution to TOC decreased in the laboratory experiment also with re-wetting intensity and in the field experiment microbial sugars in contribution to TOC (Figure Vb) significantly correlated positively with the gravimetric water content at least in the O layer and A horizons (L horizon $R^2 = 0.41$, O horizon $R^2 = 0.61$, $p < 0.05$; A horizon $R^2 = 0.81$, $p < 0.01$ and B horizon $R^2 = 0.32$). On the one hand chemical alteration and stabilization was also responsible for decreasing microbial sugars with the treatment. On the other hand Muhr et al. (2008; 2009) reported that during the drying phase explicit smaller CO_2 -fluxes were measured at the treatments than at the control, therefore the microbial activity and consequently the production of the microbial sugars in the soil was reduced by

drying.

4.2.3 Phospho lipid fatty acids (PLFA)

The soil microbial biomass was probably reduced during drying, however just with the most intensive re-wetting a significant growth and therefore recovery of soil microbial biomass could be observed. Soil drying and re-wetting events produced a significant stress to the soil microbial community (Fierer and Schimel, 2002) and a rapid change in soil water potential associated with re-wetting may cause microbes to undergo osmotic shock. After re-wetting the survived micro-organisms can multiply rapidly, the length of fungal hyphae and bacterial biomass increase (Jager and Bruins, 1974) due to increased nutrient availability, on the one hand the biomass C from the death of a portion of soil biota (Jenkinson and Powlson, 1976) and on the other hand the non-biomass C before occluded in aggregates (Vangestel et al. 1992). It is possible that this effect counteract any correlation of the total concentrations of PFLA (Figure VIa) with the water content (L horizon $R^2 = 0.13$, O horizon $R^2 = 0.37$, A horizon $R^2 = 0.00$ and B horizon $R^2 = 0.09$), which is in line with literature data (Wilkinson and Anderson, 2001), that the sum of the microbial community were not affected by the moisture regime.

Even if the sum of microbial community were hardly affected through the drying and re-wetting, the moisture regime had an effect on the soil microbial community structure. The gram+ bacteria and actinomycetes were reduced during water stress, the gram- bacteria, fungi and Protozoa were stimulated during the drying period in the L horizon or organic layer. Rapid changes in soil water potential may also favor bacteria and fungi which have thicker, more rigid cell walls and compatible solutes that enhance osmoregulatorly capabilities (Schimel et al., 1999). Wilkinson et al. (2002) reported that a warmer and drier climate increased the dominance of fungi, while a cooler and moister climate favoured the dominance of bacteria, therefore fungi were more negatively affected during the drying-rewetting

experiment than bacteria at least in the L horizon. In the O and mineral horizons the ratio fungi to bacteria changed independently from the moisture regime (O horizon $R^2 = 0.29$, A horizon $R^2 = 0.12$ and B horizon $R^2 = 0.03$). However, in the laboratory experiment due the more intensive re-wetting, fungi were stronger damaged and leached from the organic layer in comparison to the bacteria (Figure VIb). This could counteract any significant correlations. Thus the water content must be considered on future investigations of the community structure of the PLFA.

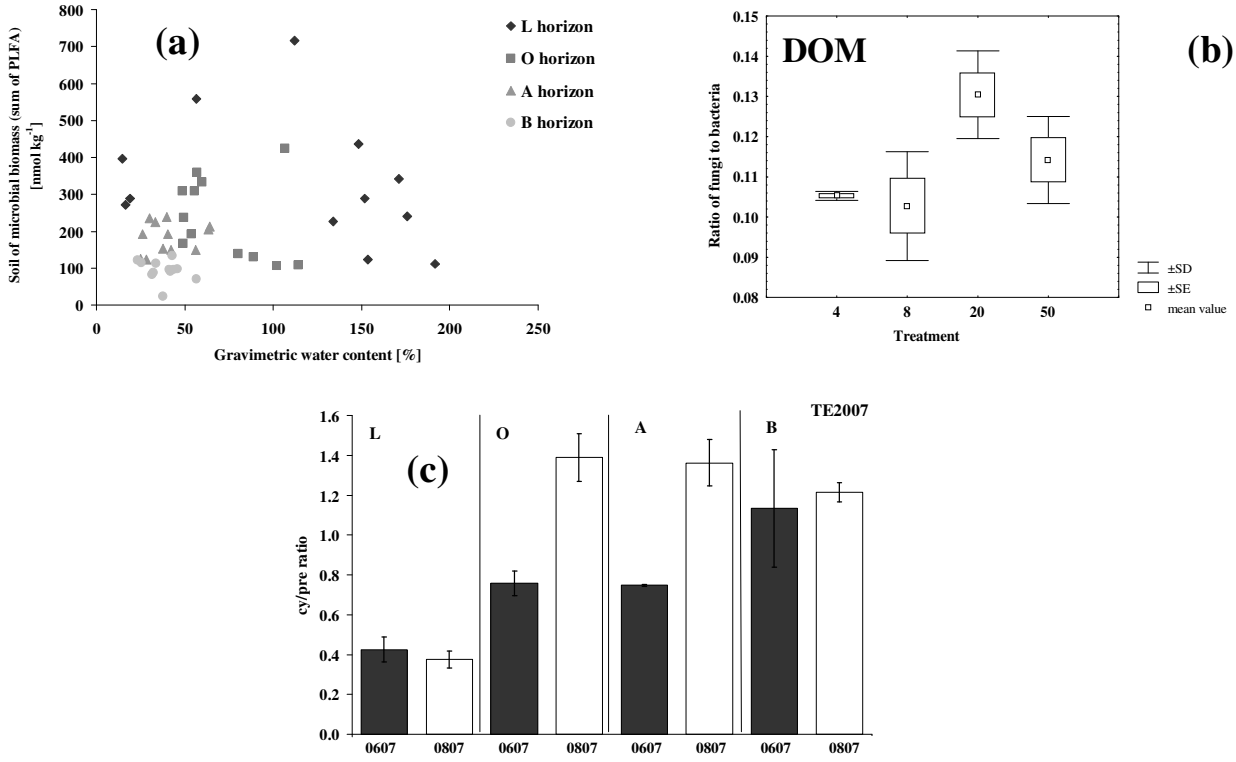

Figure VI: (a) Correlation between the gravimetric water content [%] and sum of microbial biomass [nmol kg^{-1}] in the L horizon, organic layer (O), A and B horizon. (b) ratio of fungi to bacteria in soil solution from the O horizons of the control (4 mm), 8, 20 and 50 mm treatments with added solution of the third drying and wetting cycle, (c) changes of the cy/pre ratio [(cy17:0+cy19:0)/(16:1w7c+18:1w7c)] between 12th of June (0607) and 13th of August 2007 (0807) at the throughfall exclusion plots (TE).

The increasing cy/pre ratio [(cy17:0 + cy19:0)/(16:1w7c+18:1w7c)] (Kieft et al., 1997) suggested that the microbes suffered from water stress only in the O layer and A horizon (Figure VIc). This agrees with the sum of the microbial sugars, which also correlated with the water content in the O and A horizons. In the B horizon, where soil moisture and

temperature become less variable, the microbes are more affected by carbon limitation than by climatic parameters (Fierer et al., 2003) and microbes in the L horizon may adapt to stress they experience regularly (Wilkinson and Anderson, 2001).

5 Conclusions

Repeated moderate drought followed by intensive re-wetting and frost in a Norway spruce forest did not influence TOC stocks and stable SOM pools (lignin). However, intensive frost slightly enhanced lignin mobilization in the O layer and the translocation into the B horizon. The decrease of extractable plant sugars in the organic horizons and mineral soil do not concur with the reduction of C mineralization. Therefore, for the disappearance of plant and microbial sugars upon soil freezing or drying we postulate chemical alterations of sugar molecules leading to SOM stabilization. Further studies are required to understand the mechanisms of these modifications and to examine whether these changes affect only the extraction of sugars or also the stabilization of the SOM pool. The soil microbial biomass (sum of PLFA) was neither affected by soil frost nor by soil drying. However, the increasing physiological or nutritional stress of the soil microbial biomass in the spruce forest soil changed the microbial community structure. These changes under changing climate could have effects on the C cycle of spruce forests, for instance enhanced lignin degradation by enhanced abundance of fungi leading to SOM destabilization in the longer term.

Acknowledgements

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the Research Unit 562 “Soil processes under extreme climatic conditions” (BO 1741/3-2).

Contributions to the included manuscripts

In the here presented cumulative dissertation, four studies are presented. I contributed to them by preparing all manuscripts including artwork and by putting the results in the scientific context. All biomarker analyses were gathered in my own work. The authors listed on the different manuscripts contributed approximately as follows:

Study 1:

Schmitt, A., Glaser, B., Borken, W., Matzner, E., 2008. Repeated freeze-thaw cycles changed organic matter quality in a temperate forest soil. Journal of Plant Nutrition and Soil Science, 171, 707-718.

A. Schmitt: 60 % (field work, laboratory work, discussion of results, manuscript preparation)

B. Glaser: 20 % (field work, discussion of results, comments to improve the manuscript)

W. Borken: 5 % (field work, discussion of results, comments to improve the manuscript)

E. Matzner: 5 % (discussion of results, comments to improve the manuscript)

Study 2:

Schmitt, A., Glaser, B., Borken, W., Matzner, E., 2010. Organic matter quality of a forest soil subjected to repeated drying and different rewetting intensities. European Journal of Soil Science, 61, 243-254.

A. Schmitt: 60 % (field work, laboratory work, discussion of results, manuscript preparation)

B. Glaser: 5 % (field work, discussion of results, comments to improve the manuscript)

W. Borken: 20 % (field work, discussion of results, comments to improve the manuscript)

E. Matzner: 5 % (discussion of results, comments to improve the manuscript)

Study 3:

Schmitt, A. and Glaser, B., in press 2011. Organic matter dynamics in a temperate forest as influenced by soil frost. Journal of Plant Nutrition and Soil Science.

A. Schmitt: 80 % (field work, laboratory work, discussion of results, manuscript preparation)

B. Glaser: 20 % (field work, discussion of results, comments to improve the manuscript)

Study 4:

Schmitt, A. and Glaser, B., 2011. Organic matter dynamics in a temperate forest soil following enhanced drying. Soil Biology and Biogeochemistry, 43, 478-489.

A. Schmitt: 75 % (field work, laboratory work, discussion of results, manuscript preparation)

B. Glaser: 25 % (field work, discussion of results, comments to improve the manuscript)

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Study 1

Repeated freeze/thaw cycles changed organic matter quality in a temperate forest soil

Andrea Schmitt [(a)], Bruno Glaser [(a,b*)], Werner Borken [(c)] Egbert Matzner [(c)]

[(a)] Soil Physics Department, University of Bayreuth, D-95440 Bayreuth, Germany

[(b)] Current address: Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany.

[(c)] Soil Ecology Department, University of Bayreuth, D-95440 Bayreuth, Germany

[*] Corresponding author: bruno.glaser@uni-bayreuth.de,bruno.glaser@landw.uni-halle.de

Journal of Plant Nutrition and Soil Science

Published 2008

Volume 171, Issue 5

Pages 707 – 718

Abstract

Under temperate climate, the frequency of extreme weather events such as intensive freezing or frequent thawing periods during winter might increase in the future. It was shown that frost and subsequent thawing may affect the fluxes of C and N in soils. In a laboratory study, we investigated the effect of frost intensity and repeated freeze/thaw cycles on the quality and quantity of soil organic matter (SOM) in a Haplic Podzol from a Norway spruce forest. Undisturbed soil columns comprising organic layer and top mineral soil were treated as followed: Control (+5 °C), frost at –3 °C, –8 °C and –13 °C. After a two-week freezing period, frozen soils were thawed at +5 °C and irrigated with 80 mm water at a rate of 4 mm per day. Lignin contents were not significantly affected by repeated freeze/thaw cycles. Phospholipid fatty acid (PLFA) contents decreased in the mineral soil and PLFA patterns indicate that fungi are more susceptible to soil frost than bacteria. Amounts of both plant and microbial sugars generally decreased with increasing frost intensity. These changes cannot be explained by increased mineralization of sugars or by leaching with DOM nor by a decreased microbial activity and thus sugar production with increasing frost intensity. Also physical stabilization of sugars due to frost-induced changes in soil structure can be ruled out as sugar extraction was carried out on ground bulk soil. Therefore, the only possible explanation for the disappearance of plant and microbial sugars upon soil freezing are chemical alterations of sugar molecules leading to SOM stabilization.

Keywords: Soil organic matter quality, biomarker, lignin, sugar, microbial community, climate change, extreme weather events.

1 Introduction

With the tendency of increasing global surface temperature, intensity and frequency of soil frost may change in the future, but the direction of change at the regional scale being still subject of speculation (IPCC, 2001). A lack of snow cover or late snowfall in winter results in soil freezing that is deeper and of longer duration than when early snowfall occurs (Fitzhugh et al., 2001). Therefore, without protective snow cover, freezing can be more effective and can enter considerably deeper into the soil. Alternatively, more frequent thawing periods during winter may occur.

Soil frost might influence amount, quality and turnover of SOM (Feng et al., 2007; Henry, 2007). Important SOM constituents are primary litter components such as cellulose, hemicellulose (sugars) and lignin as well as secondary microbial products such as deoxy sugars and phospholipids (Guggenberger et al., 1995; Amelung et al., 1997, 1999; Guggenberger et al., 1999; Koegel-Knabner, 2000; Gleixner et al., 2002).

Lignin is a main component in forest litter and represents a major input of organic matter into forest soils (Ziegler et al., 1986). Lignin compounds are phenolic polymers consisting of vanillin (V), syringyl (S), and cinamyl (C) moieties found in the cell walls of all vascular plants (Hedges and Ertel, 1982). The sum of V+S+C (VSC) after alkaline CuO oxidation was adopted as an indicator of the amount of intact lignin moieties (Ziegler et al., 1986). However, a quantification of lignin in soil is not possible with this method, neither with other known analytical methods (Amelung et al., 1999), nor as a result of the complex structure of lignin in combination with other organic compounds such as cellulose. Nevertheless, the alkaline CuO oxidation method releasing phenols from reactive sites of the lignin macromolecule is a relative measure for the lignin content in soils (Otto and Simpson, 2006). Several studies revealed the potential of this method to follow changes in lignin content as induced by land use changes

(Guggenberger and Zech, 1994; Amelung et al., 1999; Glaser et al., 2000; Spielvogel et al., 2007) or climate changes (Zech and Guggenberger, 1996; Amelung et al., 1997; Rodionov et al., 1999). However, little is known on the effects of soil freeze/thaw regimes on the lignin dynamics in soils.

Non-cellulosic sugars are important SOM constituents being most abundant in root exudates and non-structural plant constituents (Derrien et al., 2004). While the pentoses arabinose and xylose are mainly plant-derived, hexoses and deoxy sugars such as fucose and rhamnose are of microbial origin (Amelung et al., 1996). Plant-DNA contains deoxy ribose but not rhamnose and fucose, the latter two being analyzed in our study as microbial markers. Advanced biodegradation of SOM shifts the composition of neutral sugars from pentoses to deoxy sugars indicating consumption of plant-derived organic matter and production of microbial compounds. The ratio of plant-to-microbial sugars is a tool to trace effects of environmental changes on SOM degradation (Oades, 1989; Guggenberger and Zech, 1994; Amelung et al., 1999; Glaser et al., 2000), but changes caused by freeze/thaw cycles have not been studied yet.

Soil frost may also affect soil microbial biomass and the community of soil microorganisms. During soil freezing, biodegradation of organic matter is slowed down but may continue at soil temperatures below 0 °C (Panikov et al., 2006). The quality of SOM has been shown to affect the nature of soil microbial composition and their reaction to disturbances such as freezing and thawing (Schimel and Clein, 1996). Soil microbial biomass is strongly affected by temperature fluctuations around the freezing point (Larsen et al., 2007). Freezing events can also enhance mineralization due to increased input of labile organic matter from dead roots and microbial necromass, by physical destruction of soil aggregates or fragmentation of fresh litter (Matzner and Borken, 2008). After thawing, the activity of surviving microorganisms increase, causing a pulse of CO_2 which is often observed in laboratory experiments (Skogland et al., 1988;

Schimel and Clein, 1996).

Soil phospho lipid fatty acid (PLFA) content is a measure for soil microbial biomass (Frostegard et al., 1991) that correlates well with methods commonly used such as substrate introduced respiration (Zelles et al., 1994; Zelles, 1999; Baath and Anderson, 2003), total aldehyde content (e.g. Zelles 1999) chloroform-fumigation extraction in mineral soils (e.g. Bailey et al. 2002) and in forest floors (Leckie et al., 2004). As PLFA are subject of rapid decomposition after cell death, they characterize the current soil microbial community (White et al., 1979; Zelles et al., 1992; Leckie et al., 2004) after removing living roots prior to analysis. Further, there are specific PLFA fatty acids for microorganisms which do not occur in plants (Frostegard et al., 1993; Cavigelli et al., 1995; Zelles, 1999). The close relationship between microbial community and processes of SOM degradation and transformation suggests that environmental stress such as freeze/thaw events shift microbial community composition. Feng et al. (2007) reported that fungal biomass was greatly reduced through freeze/thaw cycles in the laboratory while bacteria were unaffected. Also Schimel et al. (2007) stated that bacteria should me more tolerant to soil freezing because the mycelia growth is more sensitive to physical disruptions caused by soil freezing. Although fungi are vulnerable to soil freezing, they sometimes increase after prolonged freezing periods (Schimel et al., 2007).

Most studies on the effect of freeze/thaw cycles on C turnover deal with CO_2 measurements (Neilson et al., 2001; Derrien et al., 2004; Larsen et al., 2007) and most laboratory experiments used disturbed or stirred samples (Sulkava and Huhta, 2003; Borjesson et al., 2004). In this study we used undisturbed soil columns from a spruce forest to be closer to natural environmental conditions. The following hypotheses were tested: Freeze/thaw events cause changes in the composition of (i) SOM, (ii) DOM, and (iii) soil microbial biomass. Results from the same experiment on soil solution (dissolved organic carbon and nitrogen) (Hentschel et al.,

2008) and gas fluxes (CO_2, CH_4, NO_x and N_2O) are reported in this issue (Goldberg et al., 2008).

2 Materials and Methods

2.1 Laboratory Experiment

Undisturbed soil columns were taken from a Haplic Podzol (see Table 1-1 for basic soil properties) under 140-years-old Norway spruce (*Picea abies (Karst.) L.*). The experimental site (Coulissenhieb II) is located in the Fichtelgebirge mountains (Northern Bavaria, Germany) about 770 m above sea level. The annual temperature in the Fichtelgebirge is +5.3 °C and the annual precipitation is 1156 mm (Gerstberger et al., 2004). The parent material of the soil is granite. Plexiglas columns of 17 cm diameter and two different lengths were driven by hand into the soil. One set contained organic layer alone and the other set organic layer plus mineral soil in fourfold replication for each of the following treatments: Control (+5 °C), frost at –3 °C, –8 °C and –13 °C (Table 1-2). The soil columns were frozen in controlled freezers (±0.5 °C) within a few hours.

Table 1-1: Chemical properties of nine soil profiles in the Norway spruce stand at the Fichtelgebirge (n=9). (CECeff = effective-cation-exchange capacity; BS = base saturation)

	Depth	**pH**		**C**	**N**	**C:N**	**Ca^{2+}**	**Mg^{2+}**	**Na^{+}**	**K^{+}**	**Al^{3+}**	**H^{+}**	**CEC_{eff}**	**BS**
	cm	H_2O	$CaCl_2$	–[%] —			——————— [$mmol_c\ kg^{-1}$] ———————							%
O_a	5	4.0	3.3	18.0	1.0	17.7	94.4	7.6	1.4	3.4	74.1	8.0	197	54
Ah	-5	4.3	3.4	7.4	0.4	19.2	60.9	3.6	1.0	1.9	66.9	7.9	145	47
Bh	-12	4.3	3.4	5.5	0.3	19.7	70.9	2.3	1.1	1.3	97.6	4.0	185	41
Bs	-18	4.6	3.7	3.4	0.2	20.5	30.1	1.1	0.9	1.0	87.8	1.3	124	27
Bw	-55	4.6	4.1	1.3	0.1	12.3	3.7	0.2	1.1	1.0	41.3	0.4	48	12
Bw/C	<-55	4.5	4.0	0.4	0.0	8.3	2.7	0.2	3.0	1.1	35.3	0.2	43	16

After two weeks of freezing, the columns were thawed in a +5 °C climate room. All columns were thermally isolated from lateral thawing. Within 8 days, all columns were completely thawed and then irrigated during 20 days at a daily rate of 4 mm with an artificial solution corresponding to natural precipitation (Matzner et al., 2004). The freezing/thawing/

irrigation cycle was repeated three times with the same columns under the same conditions.

Table 1-2: Experimental design

Parameter Freezing (14 days) [°C]	Control unfrozen +5	I -3	II -8	III -13
Thawing temperatur [°C]	+5	+5	+5	+5
Period of thawing [days]	7	7	7	7
Intensity of raining [mm/day]	4	4	4	4
Amount of raining [mm]	80	80	80	80
Period of raining [days]	20	20	20	20

→ Total amount of raining ~ 1800 ml/column

2.2 Sampling

Soil solution was sampled in 5 days intervals during the irrigation period. Percolates were filtered through 0.45 µm cellulose-acetate-membrane filters (Schleicher & Schüll, Dassel, Germany). For biomarker analyses, mixed solutions were prepared combining 60% of total percolate amounts from individual sampling dates of the third freezing cycle. Composite DOM samples were frozen at –30 °C and freeze-dried prior to analysis.

After the three freeze/thaw cycles, soil columns were separated into O, A and B horizons. The organic horizon was cut into small pieces, mixed and the mineral horizons were sieved < 2 mm. For PLFA analysis, an aliquot of fresh samples was kept frozen at –20 °C. For sugar and lignin analyses, the samples were dried at 40 °C and ground. For every horizon, the gravimetric water contents (dried at 105 °C) and the concentrations of total organic C (TOC) and total N (N) were analyzed using a Vario EL elemental analyzer (Elementar, Hanau, Germany).

2.3 Lignin analysis

The samples were oxidized with a modified alkaline CuO to release lignin-derived phenols (modified after Hedges and Ertel, 1982). Teflon-lined bombs (20 mL) were loaded with an equivalent of dry soil corresponding to 25 mg TOC (10 mg C of freeze-dried material), 300 – 400 mg CuO, 50 – 100 mg ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$], 50 mg glucose, 15 mL of 2 M NaOH, 25 µg ethyl vanillin (Fluka Chemie AG, 89555 Steinheim, Germany) in 1 mL 2 M NaOH (internal standard) and a small stainless steel ball. The teflon vessels were heated for 2 h at 170 °C on a platform shaker. After cooling to room temperature, the liquid was decanted into brown glass centrifuge tubes, the residue was washed with de-ionized water and centrifuged. The supernatant was acidified to pH 1.8 – 2.2 using and kept at room temperature in the dark (1 h) to precipitate humic acids being separated by centrifugation. C_{18} – columns (Bakerbond spe^{TM} Octadecyl (C_{18}), J. T. Baker, reversed phase material, filling volume 3 mL, particle size 5 µm) were conditioned with methanol, ethyl acetate and de-ionized water. An aliquot of 40 mL (100 mL of the DOM samples) of the solution were put onto the columns and were eluted 9 times with 0.5 mL ethyl acetate, being concentrated subsequently by rotary evaporation, transferred with 25 µL phenyl acetic acid (recovery standard, Fluka Chemie AG, 89552 Steinheim, Germany) in 1 mL methanol to 2 mL glass reaction vials and dried under nitrogen gas.

For derivatization, the CuO oxidation products were re-dissolved with 100 µL pyridine p. a. (Merck, 64271 Darmstadt, Germany) and derivatized by reaction with 200 µL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature. For quantification, derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). Chromatographic column was a fused silica capillary column SPB 5 (nominal length 30 m, nominal diameter 0.25 mm and nominal film thickness 0.25 µm, Supelco, USA). The GC-FID

operating conditions were as follows: temperature held at 100 °C for 0.5 min, increased at a rate of 15 °C min^{-1} to 250 °C, held for 6 min and increased at a rate of 30 °C min^{-1} to a final isothermal step at 300 °C for 7 min. Helium (99.996% purity, Riessner, Lichtenfels, Germany) was used as carrier gas with a constant flow at 0.8 mL min^{-1}. Using an Agilent 6890 auto sampler, 3 µL of sample were injected with a split ratio of 1:40 and the injector temperature set at 300°C.

2.4 Sugar analysis

Plant- and microbial derived sugar extraction from soil and freeze-dried material was carried out according to a modified Amelung et al. (1996) method. Samples containing 8 mg TOC (6 mg TOC of freeze-dried material) and 80 µg Myo-Inositol in de-ionized water as internal standard were hydrolyzed with 10 mL of 4 M trifluoracetic acid for 4 hours at 105 °C. After filtration through glass fiber filters (GF 6, Schleicher & Schüll, 37586 Dassel, Germany), samples were dried using a rotary evaporator (60 hPa, water temperature 45 °C). The sample was re-dissolved in 5 mL de-ionized water and put on top of a stacked Perdolit® PAD IV (5 g, Serva, 69115 Heidelberg, Germany, conditioned with three cycles of 0.1 M NaOH, de-ionized water, propanol, de-ionized water) and Dowex® 50 WX8 cation exchange resin 4 g dry Dowex® 50 W X 8 cation exchange resin (100 – 200 mesh, Serva, 69115 Heidelberg, Germany, conditioned with 2 M NaOH, de-ionized water, 2 M HCl, de-ionized water) which were washed 5 times with 10 mL de-ionized water, collected and freeze-dried. The residue containing the saccharides were re-dissolved in de-ionized water and transferred into 3 mL glass reaction vials which were frozen until derivatization.

Derivatization was carried out according to a modified procedure described by Gross and Glaser (2004). The frozen vials were freeze-dried and the sugars were re-dissolved in dry

pyridine p.a. (Merck KgaA, 64271 Darmstadt, Germany) containing 10 µg 3-O-Methylglucose (3-OM) (Sigma-Aldrich-Chemie, 82024 Taufkirchen, Germany) as recovery standard and in 450 µL dry pyridine p.a. containing 45 µg methyl boric acid (MBA) (Sigma-Aldrich, 82024 Taufkirchen, Germany). The samples were heated at 60 °C for one hour and diluted with ethyl acetate after cooling. The derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). The column was a fused silica capillary column SPB 5 (nominal length 60 m, nominal diameter 0.25 mm and nominal film thickness 0.25 µm, Supelco, USA). The GC-FID operating conditions were as follows: initial temperature held at 90 °C for 2 min, increased at a rate of 10 °C min^{-1} to 110 °C held for 23 min, increased at a rate of 11 °C min^{-1} to 145 °C held for 8 min and increased at a rate of 60 °C min^{-1} to a final isothermal step at 300 °C held for 11 min. Helium (99.996% purity, Riessner, Lichtenfels, Germany) was used as carrier gas with a constant flow at 2.0 mL min^{-1}. Four µL of sample were injected splitless (splitless time one min) with an Agilent 6890 auto sampler and the injector temperature was set at 310 °C.

2.5 Phospho lipid fatty acids (PLFA) analysis

Phospho lipid extraction from soil and freeze-dried DOM was carried out according to a modified Frostegard et al. (1991) method. To 5 g fresh soil or freeze-dried DOM corresponding to 10 mg TOC, 18 mL extraction solution was added [1 : 2 : 0.8 chloroform : methanol : citrate buffer solution (6.3 g citric acid monohydrate in 200 mL de-ionized water and with potassium hydroxide pellets adjusted to pH 4.0), and shaken for 2 hours at 225 rpm. After centrifugation (4000 rpm for 20 min) the supernatant was transferred into separating funnels. The residue was washed with 5 mL extraction solution, shaken for one hour at 225 rpm and after centrifugation the supernatant was also transferred into the separating funnel. Subsequently, 15 µg (10 µg in

case of freeze-dried DOM) PLFA 19:0 (internal standard, Biotrend, 50933 Cologne, Germany), 6.2 mL chloroform and 6.2 mL citrate buffer were added and shaken for 15 min. After separation over night, the organic extract (lower phase) was transferred into 25 mL conical flasks and dried using a rotary evaporator (300 hPa, water temperature 35 °C). The samples were re-dissolved in chloroform and separated over glass columns filled with silica gel into neutral (by elution with 5 mL chloroform), glyco- (by elution with 20 mL acetone) and phospho- (polar) lipids (by elution with 2 x 10 mL methanol). The methanol extract was dried using a rotary evaporator (100 hPa, water temperature 35 °C), transferred into 4 mL glass reaction vials with methanol and dried under a stream of nitrogen.

Fatty acid methyl esters (FAME) were prepared from free PLFA using a strong acid methylation. For this aim, the PLFA were re-dissolved in 0.5 mL of 0.5 M NaOH in methanol. The samples were put in an ultrasonic bath (Bandelin Sonorex Super RK 103H) for 10 min for better solubility and heated at 100 °C for 10 minutes and cooled to room temperature. After the addition of 0.75 mL boron trifluoride in methanol (concentration of BF_3: 13 – 15%; Fluka, Seelze, Germany) the samples were heated at 80 °C for 15 minutes and cooled to room temperature again. After addition of 0.5 mL saturated NaCl solution, the samples were shaken 3 times for 30 seconds with 1 mL hexane. The hexane (upper) phase was pipetted into another reaction vial and dried under a stream of nitrogen. The PLFA were re-dissolved in 10 µL (5 µL for DOM) 13:0 FAME (Sigma-Adrich, 82024 Taufkirchen, Germany) in toluene as recovery standard and 490 µL (95 µL in case of DOM) toluene and transferred into GC auto sampler vials. The derivates were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). The column was a fused silica capillary column SPB 5 (nominal length 60 m, nominal diameter 0.25 mm and nominal film thickness 0.25 µm, Supelco Park, USA). The GC-FID operating conditions were as follows: initial temperature 80 °C held for 1 min, increased at a

rate of 7 °C min^{-1} to 180 °C, increased at a rate of 1.3 °C min^{-1} to 195 °C and held for 5 min, increased at a rate of 1.3 °C min^{-1} to 220 °C and increased at a rate of 60 °C min^{-1} to a final isothermal step at 300 °C held for 5 min. Helium (99.996% purity, Riessner, Lichtenfels, Germany) was used as carrier gas with a constant flow at 2.4 mL min^{-1}. Four µL of sample were injected with an Agilent 6890 auto sampler in splitless mode at the injector temperature set at 300°C.

Terminal-branched saturated PLFA (a15:0, i15:0, i16:0, i17:0, a17:0) were considered as markers for gram-positive bacteria and mid chain-branched saturated PLFA (10Me16:0, 10Me17:0, 10Me18:0) were associated with actinomycetes. Typical for gram-negative bacteria are monounsaturated PLFA (16:1ω7c, 18:1ω7c) and cyclopropyl saturated PLFA (cy17:0, cy19:0). Short or odd-chain saturated PLFA (14:0, 15:0, 16:0, 17:0, and 18:0) were considered non-specific bacterial makers because they are present in all microbial groups. Typical markers for fungi are PLFA 18:2ω6,9, 18:1ω9c and 16:1ω5c (Stahl and Klug, 1996; Zelles, 1999; Myers et al., 2001; Ruess et al., 2002; DeForest et al., 2004; Waldrop et al., 2004; McMahon et al., 2005). The ratio of fungal/bacterial PLFA (fungi/sum of gram-positive bacteria + actinomycetes + gram-negative bacteria) can be used as indicator for nutritional stress (Fierer et al., 2003b).

2.6 Statistical analysis

Statistical analyses were carried out using STATISTICA 6.0. Microbial community structure differences were evaluated by principal component analysis (PCA). According to the Kaiser criteria, the number of factors (principal components) with an Eigen value greater than one was selected (Stoyan et al., 1997). Cluster analysis was performed with the factor values received from the PCA to create similar microbial community groups. As distance unit the Euclidian distance was selected. Using the elbow criteria, the number of clusters was selected in that way

that a further fusion would have led to rapid increase of the error square sum (Janssen and Laatz, 1999). Differences between different treatments were evaluated using a one-way ANOVA followed by the Tuckey-Honest post-hoc test.

3 Results and Discussion

3.1 Carbon balance

The investigated C pools and fluxes generally decreased in the order TOC >> neutral sugars > lignin >> mineralized CO_2 > DOC > PLFA (1- 3). While no significant changes in SOC contents could be detected, SOM pools were partly affected after three freeze/thaw cycles (Table 1-3). The content of plant and microbial sugars decreased more than all other investigated SOM pools both in the organic layer alone and in combined organic layer and mineral soil columns (Table 1-3). Total PLFA amounts decreased significantly ($p < 0.05$) in all frost-treated soil columns compared to the control, while in organic layer no significant decrease of PLFA contents was observed (Table 1-3). No significant changes in lignin amounts with increasing frost intensity were observed (Table 1-3).

The total SOC content of organic layer plus mineral horizons was about twice as high compared to the organic layer (Table 1-3) alone while CO_2 release was about the same in the two different soil column experiments indicating that mineralization took only place in the organic layer. On the other hand, DOC export was three to four times higher from the organic layer alone compared to organic layer plus mineral soil, indicating a significant DOM retention in the mineral soil (Table 1-3). While repeated soil freezing/thawing had little effect on SOC quantity, individual SOM pools and DOM were affected (Table 1-4) which will be discussed for individual components in the following.

Table 1-3: Carbon balance of the column experiment (a) organic layer alone (O), (b) organic layer + mineral horizons (O+M) after three freeze-thaw cycles. Results were scaled from weight-based to volumne-based vial soil density for better comparability with field studies and literature date.

(a)	O [g m^{-2}]	+5°C	SE	-3°C	SE	-8°C	SE	-13°C	SE
	SOC	6628	± 427	7087	± 804	6238	± 628	7285	± 350
	CO_2-C[a]	54.6	± 2.5	46.1	± 2.0	43.2	± 2.1	54.0	± 5.4
	DOC[b]	10.6	± 2.0	9.2	± 0.8	13.0	± 2.7	12.5	± 1.2
	Lignin (VSC)	365.9	± 31.6	265.7	± 66.7	468.8	± 62.3	309.8	± 33.5
	Total sugar	687.2	± 62.1	364.8	± 66.2	398.9	± 27.8	255.2	± 28.7
	PLFA	2.8	± 0.5	5.1	± 1.4	4.2	± 2.4	2.9	± 0.7

(b)	O + M [g m^{-2}]	+5°C	SE	-3°C	SE	-8°C	SE	-13°C	SE
	SOC	13079	± 884	11729	± 1106	14089	± 550	11581	± 480
	CO_2-C[a]	54.9	± 4.3	63.8	± 7.8	61.6	± 2.7	63.3	± 5.9
	DOC[b]	3.1	± 0.3	3.0	± 0.3	3.9	± 0.3	3.7	± 0.2
	Lignin (VSC)	529.1	± 55.5	390.2	± 74.4	677.5	± 54.9	507.7	± 34.5
	Total sugar	1060.3	± 103.2	514.1	± 85.0	614.6	± 86.2	388.0	± 32.8
	PLFA	46.4	± 3.6	26.3	± 5.0	16.7	± 6.3	34.9	± 12.7

SE = standard error of the mean

[a] cumulative CO_2 emission during three thawing period (Goldberg et al., 2008)

[b] cumulative DOC leaching during three thawing period (Hentschel et al., 2008)

3.2 Lignin phenols

The sum of lignin phenols concentration (VSC) generally decreased in the order O layer < A horizon < B horizon (Figure 1-1a) while the lignin contribution to SOC decreased in the order O layer < A horizon ≈ B horizon (Figure 1-1b). Frost intensity had no effect on lignin concentration in soil (Figure 1-1a), while the two most intensive frost treatments (-8 and -13°C) induced slightly higher lignin contribution to SOC (Figure 1-1b). In O layer percolates, the same tendency was true for the contribution of lignin phenols to dissolved organic carbon (DOC, Figure 1-1c). Here, the most intensive frost (-13 °C) caused significantly ($p < 0.05$) higher lignin contribution to DOC compared to the least intensive (-3°C) treatment. Lignin contribution to SOC ranged between 2 and 7% , similar to other acid temperate forest soils (Guggenberger et al., 1998; Glaser

et al., 2000; Koegel-Knabner, 2000; Spielvogel et al., 2007).

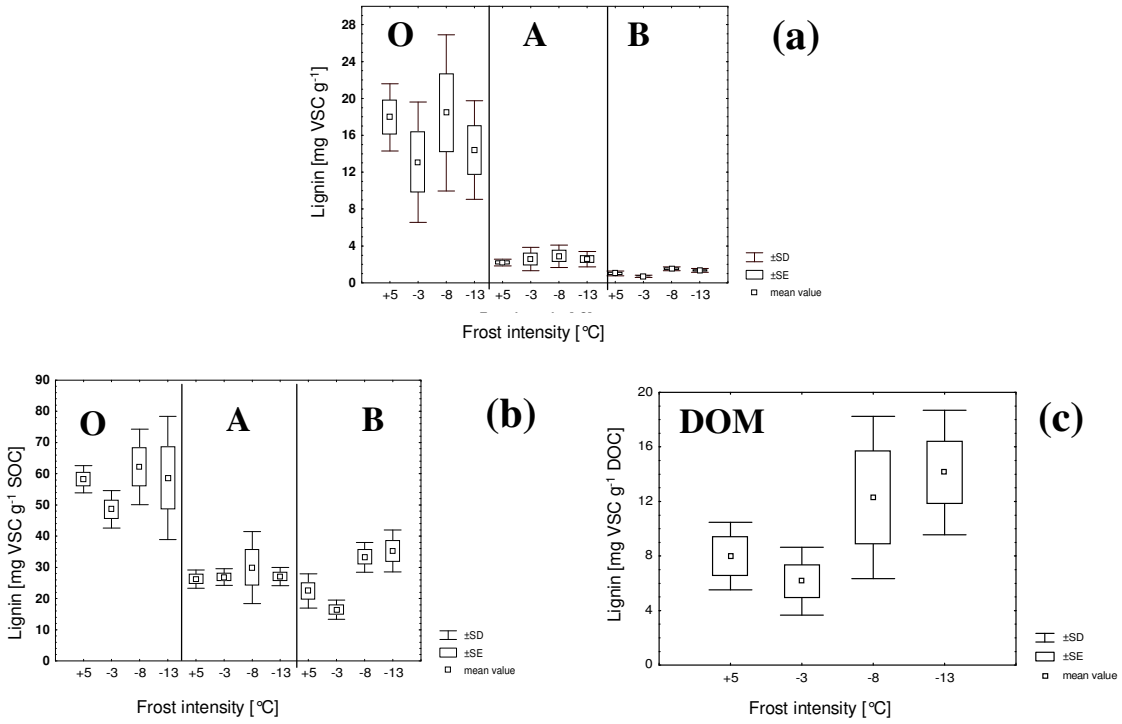

Figure 1-1: Lignin (sum of VSC) concentration (a) in soil and (b) contribution to SOC and (c) to DOC leached through O horizons only.

While lignin concentrations generally decreased with increasing soil depth (Figure 1-1a), acid to aldehyde ratios of vanillin increased in the same direction (data not shown), indicating advanced lignin decomposition with increasing soil depth which is in agreement with other studies on lignin distribution in forest soils (Koegel, 1986; Ziegler et al., 1986).

Little effects of freeze/thaw cycles were observed on amount and distribution of lignin (Figure 1-1). Only the most severe frost intensities (< -8 °C) caused slightly increased lignin concentrations in the B horizon and in DOM (Figure 1-1). These increases were mainly caused by increasing concentrations of syringyl units (both aldehyde and acid) known as less stable compared to vanillyl units (Koegel-Knabner, 2000). Acidic lignin degradation products are typically retained in the soil due to sorption to minerals (Guggenberger and Zech, 1994; Kaiser et

al., 2004; Kawahigashi et al., 2006). However, as the cumulative CO_2 emissions and DOC fluxes were rather similar in the different treatments (Table 1-3), biological lignin degradation in the topsoil and subsequent leaching of lignin degradation products cannot be responsible for the slightly increased lignin amounts in the B horizon and in DOM. Instead, soil freezing may have induced physical disruption of soil aggregates and a release of otherwise physically protected particulate SOM (Bullock et al., 1988) and microbial products (Skogland et al., 1988). Thus, intensive frost (> -8 °C) led to physical-chemical changes of SOM and to slightly enhanced lignin solubility in the litter layer as indicated by higher lignin concentrations in DOM leached through the O layer (Figure 1-1c). The lignin compounds are adsorbed to minerals in the B horizons as indicated by higher lignin concentrations in the B horizon (Figure 1-1) but lower DOC concentrations in the soil solution leached through the organic and mineral soil (Table 1-3). Also Kawahigashi et al. (2004) observed higher DOC concentrations and contribution of hydrophobic compounds such as lignin with increasing permafrost intensity (-0.1 to -2.5°C) in water samples along the Yenisei River between 69°30'N and 65°49'N latitude in Siberia which is in accordance with our results.

3.3 Plant and microbial sugars

The concentrations of the sum of plant derived sugars (arabinose + xylose, Figure 1-2a), microbial derived sugars (rhamnose and fucose, Figure 1-2b) as well as the total sugar concentration (Figure 1-2c) and their contribution to SOC (Figures 1-2d, e, and f, respectively) decreased in the order O layer < A horizon < B horizon. In the control treatment, total sugar concentrations were in the same range as reported for other soils (Amelung et al., 1996) but lower than in other German forest soils (Spielvogel et al., 2007). The latter might be due to methodological differences because we did not analyse microbial hexoses. Also decreasing sugar

concentrations with increasing soil depth is in line with literature data (Rumpel et al., 2002).

Plant and microbial sugar concentrations decreased with increasing frost intensity (Figures 1-2a and b, respectively). In the O layer and A horizon, both plant and microbial sugars concentrations and contribution to SOC decreased by a factor of about three when comparing the most intensive frost treatment with the unfrozen control (Figures 1-2a-f). In the B horizon differences were smaller, but also here decreasing sugar concentrations and contribution to TOC were observed with increasing frost intensity (Figures 1-2a-f). Decreasing microbial sugars with increasing frost intensity indicate reduced microbial activity in the same direction corroborating decreasing mineralization rates with increasing frost intensity in the 3rd cycle of the experiment (Goldberg et al., 2008).

If microbial activity is reduced under frost, plant sugar concentrations should be similar to the controls, but this was not the case (Figure 1-2a). Instead, also plant sugars decreased with increasing frost intensity. As already mentioned, increased mineralization can be ruled out explaining this phenomenon because the decrease of sugar concentrations is much higher as could be explained by increased mineralization as indicated by differences in CO_2 emissions (Table 1-3).

The ratio of plant (arabinose and xylose) to microbial sugars (fucose and rhamnose) remained constant in the O layer and A horizon but increased in the B horizon, indicating similar processes for plant and microbial sugar losses in O layer and A horizon but different processes in the B horizon. In the latter case the decrease of plant sugars was less pronounced and the ratio of plant to microbial sugars increased with frost intensity. (Figure 1-2g).

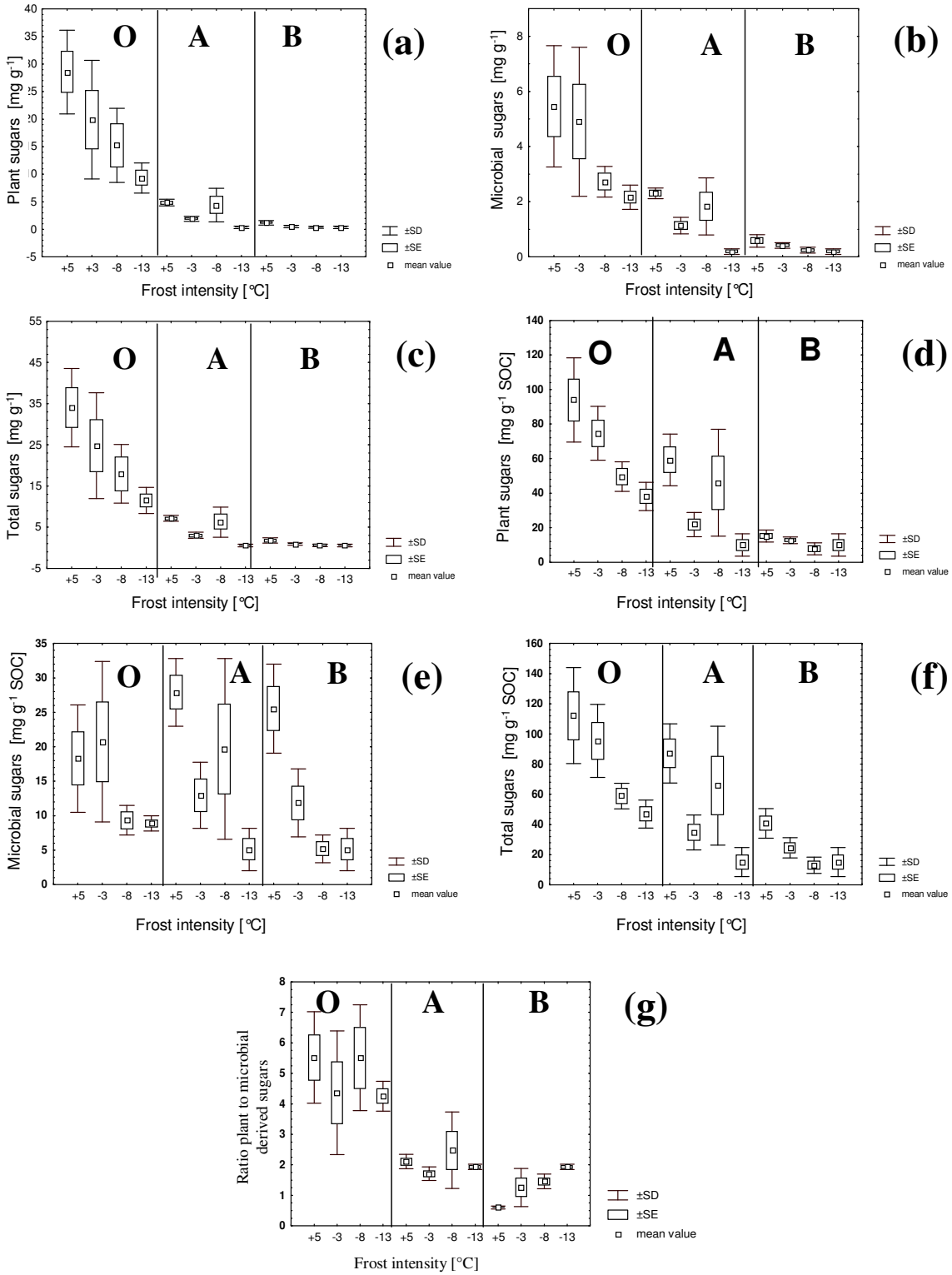

Figure 1-2: (a) Plant, (b) microbial and (c) total sugar concentrations in soil, and their contribution to TOC [(d), (e) and (f), respectively] and (g) the ratio of plant to microbial sugars in soil columns after different frost intensity.

Another process responsible for decreasing sugar concentrations with increasing frost

intensity could be could be the mobilization and translocation of the sugars with DOM. This is supported by the observation stated in the literature that DOM formed during winter and spring is dominated by carbohydrate-rich material from disrupted fresh microbial and plant debris (Kawahigashi et al., 2003). However, this process cannot explain the tremendously decreasing sugar concentrations with increasing frost intensity in our experiment as changes in sugar pools were at least two orders of magnitude higher than the differences in DOM fluxes leached through the O layer (Table 1-3).

Thus, physical changes of soil structure and / or organo-mineral stabilization of sugars might be responsible for the changing amounts of extractable sugars. For instance, it is well known that freeze/thaw events affect soil structure (Tisdall and Oades, 1982), which influence C availability, aeration and water and nutrient movement, all of which have important effects on quantity and quality of SOM.

Additionally, it cannot be completely ruled out that the analytical method itself is responsible for the observed changes in sugar concentrations as direct contact of extraction solution with SOM is necessary for carbohydrate monomer release. Physical-chemical changes of soil structure can theoretically change also sugar extractability from soils although the applied method was also able to quantify organo-mineral stabilization of sugars in soil (Spielvogel et al., 2007). Nevertheless, if lower sugar extractability would be responsible for our findings, the ecological consequences would be the same as for a decrease of sugar concentrations due to soil structure changes, namely, transformation of a labile into a stable SOM pool.

A further possible explanation is enhanced plant and microbial sugar production in the control treatment and the weakly frozen soil columns compared to the more severe frost treatments e.g. by exo-enzymes being responsible for the observed differences in sugar concentrations. However, as sugar concentrations of an air-dried soil were higher than of the

same soil (not air-dried) kept in a refrigerator (+4 to +8 °C, 4 weeks) or a freezer (-18 °C, 4 weeks) sugar production in less frozen soil can be ruled out as explanation for decreasing sugar concentrations with increasing frost intensity (Figure 1-3). Therefore, as both reduced mineralization and physical stabilization can be ruled out, chemical alteration and stabilization is responsible for sugar decrease with increased soil frost intensity.

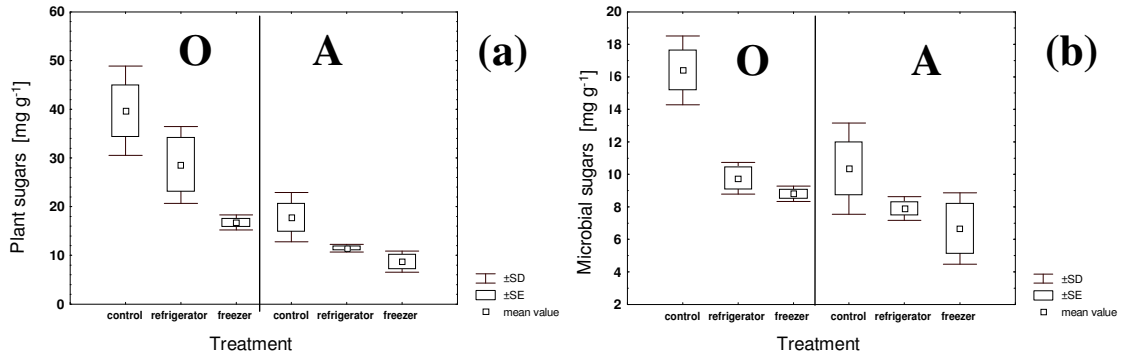

Figure 1-3: (a) Plant and (b) microbial sugar concentrations in organic layer (O) and mineral soil (A) after air-drying, cooling (4 weeks at +4 to +8 °C) and freezing (4 weeks at -18 °C).

3.4 Phospho lipid fatty acids (PLFA)

The sum of PLFA concentrations in soil was in the same order of magnitude for all investigated soil horizons and frost treatments (Figure 1-4a). Only the A horizon of the controls had higher PLFA concentrations but the variation among replicates was also higher (Figure 1-4a). On the other hand, PLFA contribution to SOC was higher in mineral soil horizons compared to the organic layer in the control while for the frost treatments microbial PLFA contribution to SOC was comparable for organic and mineral horizons (Figure 1-4b). PLFA contribution to TOC in our study was comparable to data from *Pinus sylvestris*, *Picea abies* and *Betula pendula* forest soils in Finland (Priha et al., 2001). Interestingly, the same authors also found higher microbial biomass contribution to TOC as measured by both chloroform fumigation extraction and PLFA

concentrations in mineral soil horizons compared to organic layer. On the other hand, microbial activity was higher in the organic layer compared to the mineral soil as measured by respiration indicating that soil microbial biomass in mineral soil horizons is less active than in the organic layer.

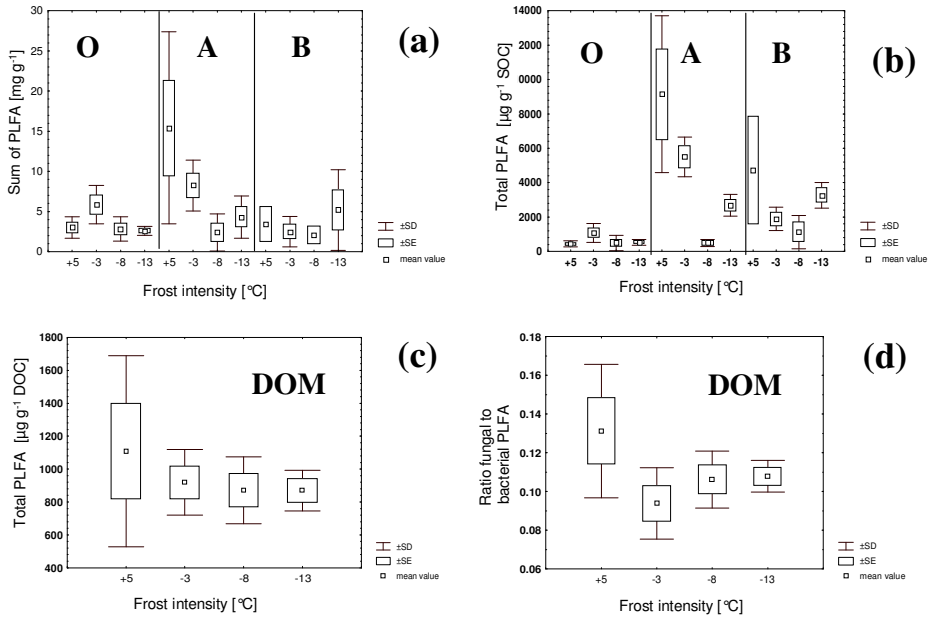

Figure 1-4: Sum of PLFA concentrations (a) in soil and (b) in contribution to SOC and (c) in DOC leached through O layers only and (d) the ratio of fungal to bacterial PLFA in DOM.

A tendency of decreasing PLFA concentrations with increasing frost intensity was observed in the soil mineral horizons (Figure 1-4b, Table 1-4). In the A horizon, this effect was statistically significant ($p < 0.05$) for PLFA 15:0, 16:0, 17:0, 18:0, a15:0, cy19:0, 18:2ω6,9 and 18:1ω9c from the more intensive frost treatment (Table 1-3). PLFA concentrations in DOM were one order of magnitude lower compared to SOM and a slight tendency ($p > 0.05$) of decreasing PLFA contents with increasing frost intensity could be observed (Figure 1-4c). The ratio of fungal/bacterial PLFA slightly decreased when frost was applied (Figure 1-4d). Soil microbial community may react fast to disturbance and might therefore be a sensitive indicator for soil changes (Kaur et al., 2005). However, in contrary to plant and microbial sugars, our freeze/thaw experiment revealed

little effects on soil microbial biomass as assessed by PLFA concentrations: Increasing frost intensity had no significant effect on total PLFA concentrations (Figure 1-4a), only PLFA contribution to SOC in the A horizon decreased with increasing frost intensity (Figure 1-4b). Koponen et al. (2006) observed that soil microbial community structure and biomass analysed with PLFA were not affected by freeze/thaw events. The more or less constant PLFA concentrations can be explained by the fact that microbial processes are still active in cold (0 to 5°C) and even frozen soils (Brooks et al., 1998). The critical temperature for active respiration and therefore growth is assumed to be between -7 °C and -5 °C (Brooks et al., 1998). The underlying process is a lack of free water limiting heterotrophic respiration (Schimel and Clein, 1996; Brooks et al., 2005). Also Neilsen et al. (2001) supposed that microbes in the upper soil horizons are adapted to freezing stress. Lipson et al. (2000) suggested that microbes may adapt to stress they experience regularly, such tolerance has been observed for example in alpine tundra or in Antarctic soils. On the other hand, 40% lower soil respiration was observed during the third frost cycle compared to unfrozen control (Goldberg et al., 2008). Also ^{14}C measurements revealed significantly reduced heterotrophic soil respiration of frozen soil (Muhr et al., 2008).

In the A horizon, the more intensive frost treatment induced significantly ($p < 0.05$) lower concentrations of PLFA 15:0, 16:0, 17:0, 18:0, a15:0, cy19:0, 18:2ω6,9 and 18:1ω9c (Table 1-4), indicating that especially gram-negative bacteria and fungi are sensitive to frost in mineral soil. Also in DOM, a tendency of decreased fungal to bacterial PLFA ratio was observed in the frost treatments (Figure 1-4d). Thus, the frost treatment seems to affect fungi more than bacteria, a conclusion that is in accordance with literature findings: Feng et al. (2007) observed quick recovery of soil bacteria but a decrease of fungi after 8 freeze/thaw cycles at -15 °C for 1 day on disturbed Chernozem samples. Also Hoegberg (2006) found decreasing fungal PLFA concentrations upon various disturbances of boreal forest ecosystems.

Table 1-4: Indiviual PLFA concentrations ± SD [µg (g TOC)$^{-1}$].

PLFA [µg (g C)$^{-1}$]		O horizon				A horizon				B horizon			
		+5°C SD	-3°C SD	-8°C SD	-13°C SD	+5°C SD	-3°C SD	-8°C SD	-13°C SD	+5°C SD	-3°C SD	-8°C SD	
Common	14:0	9.5 ± 4.0	22.8 ± 8.5	10.2 ± 6.4	10.6 ± 3.5	201.3 ± 97.8	123.7 ± 21.5	12.5 ± 5.6	63.7 ± 19.7	77.7 ± 88.3	35.8 ± 12.3	20.4 ± 15.2	
	15:0	6.17 ± 2.31	16.64 ± 8.31	6.16 ± 4.06	6.07 ± 2.20	86.40 ± 37.09	52.22 ± 17.54	5.55 ± 2.26	22.18 ± 9.00	40.45 ± 45.53	16.89 ± 7.66	10.64 ± 9.79	
	16:0	80.58 ± 31.52	202.81 ± 111.69	86.31 ± 66.44	87.01 ± 27.93	888.56 ± 406.89	537.24 ± 190.81	51.40 ± 22.10	260.36 ± 100.12	556.03 ± 632.35	259.59 ± 87.41	150.32 ± 122.63	
	17:0	4.62 ± 2.03	10.10 ± 6.23	3.87 ± 3.05	3.53 ± 1.15	44.50 ± 18.83	24.07 ± 7.01	2.96 ± 1.08	12.21 ± 2.67	132.68 ± 172.66	34.92 ± 15.87	33.01 ± 29.96	
	18:0	27.77 ± 13.55	67.18 ± 31.80	23.32 ± 12.41	23.55 ± 7.97	226.14 ± 92.04	154.70 ± 61.85	16.56 ± 6.97	129.65 ± 41.97	198.18 ± 211.12	121.04 ± 30.07	61.92 ± 43.38	
Gram+	i15:0	48.17 ± 19.86	110.98 ± 55.99	51.55 ± 43.46	55.67 ± 18.45	959.66 ± 505.29	548.21 ± 120.20	48.38 ± 22.43	230.52 ± 102.38	410.18 ± 478.16	163.24 ± 61.42	89.41 ± 64.93	
	a15:0	9.07 ± 4.14	21.02 ± 11.83	12.05 ± 13.36	14.67 ± 5.07	288.23 ± 161.88	145.27 ± 23.69	12.31 ± 4.47	61.65 ± 12.75	103.88 ± 127.76	30.22 ± 13.28	22.25 ± 16.26	
	i16:0	45.10 ± 18.93	91.21 ± 48.90	45.71 ± 40.56	57.96 ± 14.03	692.88 ± 452.06	418.19 ± 219.22	38.70 ± 21.10	126.52 ± 109.91	150.59 ± 171.21	72.92 ± 29.79	47.74 ± 36.09	
	i17:0	15.74 ± 9.31	54.58 ± 30.05	15.60 ± 6.14	15.90 ± 7.33	57.73 ± 46.53	80.65 ± 69.66	4.98 ± 1.94	27.28 ± 20.13	108.02 ± 134.68	43.72 ± 14.10	26.80 ± 17.59	
	a17:0	8.32 ± 3.34	20.73 ± 10.49	10.44 ± 9.99	13.13 ± 4.19	144.35 ± 76.22	80.89 ± 37.40	6.44 ± 2.20	35.34 ± 19.12	56.44 ± 67.85	19.52 ± 8.53	13.62 ± 9.78	
Actinom-	10Me16:0	39.33 ± 16.27	75.41 ± 42.59	35.04 ± 37.64	48.46 ± 15.04	754.82 ± 449.43	415.62 ± 117.59	37.04 ± 17.96	151.38 ± 74.85	298.12 ± 365.83	91.79 ± 39.10	68.41 ± 56.15	
cetes	10Me17:0	6.88 ± 2.48	14.56 ± 7.87	7.16 ± 7.08	9.64 ± 2.83	86.55 ± 52.09	49.56 ± 24.81	4.09 ± 2.61	17.18 ± 12.69	19.60 ± 24.42	8.85 ± 3.95	4.75 ± 3.79	
	10Me18:0	9.24 ± 4.47	32.37 ± 15.72	11.14 ± 7.25	13.09 ± 4.16	163.53 ± 85.13	136.90 ± 92.20	9.67 ± 3.49	51.56 ± 36.83	136.64 ± 171.24	52.96 ± 9.01	29.44 ± 24.27	
Gram-	16:1□7c	20.82 ± 8.40	46.10 ± 24.78	22.77 ± 28.38	26.52 ± 9.08	608.49 ± 343.74	288.21 ± 72.58	25.31 ± 12.54	131.03 ± 41.13	229.60 ± 269.02	83.88 ± 46.79	36.37 ± 26.60	
	18:1□7c	15.10 ± 5.21	37.88 ± 29.67	26.31 ± 37.14	19.82 ± 8.17	592.18 ± 232.01	360.35 ± 168.41	20.65 ± 10.26	125.67 ± 29.11	388.50 ± 496.59	126.99 ± 74.99	60.75 ± 61.35	
	cy17:0	5.77 ± 2.69	12.54 ± 7.66	6.43 ± 8.58	7.11 ± 3.07	157.17 ± 124.64	74.89 ± 38.69	5.68 ± 3.24	36.19 ± 5.88	104.72 ± 132.25	27.76 ± 29.70	13.04 ± 10.14	
	cy19:0	29.61 ± 11.65	51.71 ± 31.46	35.82 ± 48.71	34.34 ± 18.33	1394.02 ± 768.93	665.35 ± 146.98	52.65 ± 26.80	288.30 ± 66.66	625.49 ± 702.77	204.16 ± 177.87	140.75 ± 147.24	
Fungi	18:2□6,9	13.96 ± 4.91	44.74 ± 25.30	17.59 ± 17.85	13.18 ± 5.23	163.34 ± 90.42	93.28 ± 38.60	6.38 ± 3.71	31.19 ± 6.70	53.33 ± 59.59	32.32 ± 10.66	12.26 ± 12.26	
	18:1□9c	39.85 ± 18.04	120.92 ± 68.78	50.82 ± 43.37	46.99 ± 17.11	654.66 ± 303.19	397.46 ± 148.37	29.30 ± 13.73	167.33 ± 43.83	405.43 ± 457.99	213.48 ± 45.67	100.14 ± 92.09	
VAM	16:1□5c	4.95 ± 2.30	12.12 ± 6.38	4.91 ± 6.13	5.67 ± 2.11	176.76 ± 127.09	74.99 ± 21.92	6.22 ± 2.75	36.54 ± 3.37	86.41 ± 109.79	28.54 ± 16.96	11.25 ± 8.08	
Protozoa	20:4□6	3.07 ± 3.75	9.15 ± 5.62	5.67 ± 3.23	5.33 ± 5.76	801.18 ± 449.65	781.43 ± 303.37	88.94 ± 20.14	671.42 ± 194.44	534.27 ± 505.29	221.00 ± 94.18	165.71 ± 167.77	
PLFA (21)	sum	443.6 ± 177.6	1075.6 ± 555.6	488.9 ± 444.3	518.2 ± 168.6	9142.5 ± 4558.9	5503.2 ± 1156.5	485.7 ± 201.1	2677.2 ± 632.8	4716.2 ± 5421.9	1889.5 ± 675.4	1119.0 ± 972.3	

5 Conclusions

Our repeated freeze/thaw experiment clearly revealed dramatic changes of labile SOM pools while the total SOC concentrations were not significantly affected. Intensive frost slightly enhanced lignin mobilization in the O layer and the translocation into the B horizon. Interestingly, soil microbial biomass was not affected by increasing frost intensity but the composition of the soil microbial community: Fungi were more negatively affected by soil frost than bacteria. Fungal hyphae may suffer from frost-induced physical breakdown. The most severe effect of repeated soil freeze/thaw was on hydrolysable plant and microbial sugars, which dramatically decreased with increasing frost intensity which might be explained by the following soil processes: i) changes in soil structure , ii) interaction of sugars with the mineral phase or iii) decreasing sugar production with decreasing frost. Further studies are required to quantify the effect of temperature to the observed changes in soil sugar concentrations.

Acknowledgements

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) within the Research Unit 562 “Soil processes under extreme climatic conditions” (BO 1741/3-2).

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Study 2:

Organic matter quality of a forest soil subjected to repeated drying and different re-wetting intensities

Andrea Schmitt [(a)], Bruno Glaser [(ab)], Werner Borken [(c*)] Egbert Matzner [(c)]

[(a)] Soil Physics Department, University of Bayreuth, D-95440 Bayreuth, Germany

[(b)] Current address: Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany.

[(c)] Soil Ecology Department, University of Bayreuth, D-95440 Bayreuth, Germany

* Corresponding author: werner.borken@uni-bayreuth.de

European Journal of Soil Science

Published 2010

Volume 61

Pages 243-254

Abstract

Extended drought periods followed by heavy rainfall may increase in many regions of the Earth, but the consequences for the quality of soil organic matter and soil microbial communities are poorly understood. Here, we investigated the effect of repeated drying and re-wetting on microbial communities and the quality of particulate and dissolved organic matter in a Haplic Podzol from a Norway spruce stand. After air-drying, undisturbed soil columns were re-wetted at different intensities (8, 20 and 50 mm per day) and time intervals, so that all treatments received the same amount of water per cycle (100 mm). After the third cycle, SOM pools of the treatments were compared with those of non-dried control columns. Lignin phenols were not systematically affected in the O horizons by the treatments whereas fewer lignin phenols were found in the A horizon of the 20 and 50 mm treatments. Microbial biomass and the ratio of fungi to bacteria were generally not altered, suggesting that most soil microorganisms were well adapted to drying and re-wetting in this soil. However, gram-positive bacteria and actinomycetes were reduced whereas gram-negative bacteria and protozoa were stimulated by the treatments. The increase in the (cy17:0+cy19:0)/(16:1ω7c+18:1ω7c) ratio indicates physiological or nutritional stress for the bacterial communities in the O, A and B horizons with increasing re-wetting intensity. Drying and re-wetting reduced the amount of hydrolysable plant and microbial sugars in all soil horizons. However, CO_2 and dissolved organic carbon fluxes could not explain these losses. We postulate that drying and re-wetting triggered chemical alterations of hydrolysable sugar molecules in organic and mineral soil horizons.

Keywords: Soil organic matter quality, biomarker, lignin, sugar, microbial community, climate change, extreme weather events.

1 Introduction

Climate models predict an increase in global surface temperature and a change in the intensity of precipitation during the current century (IPCC, 2007). During recent decades, increasing drought periods followed by heavy rainfall have been observed in many regions of the earth. Because drought stress reduces both litter production and the decay of soil organic matter (SOM), the adjustment to a new balance between these two processes has the potential to alter the global carbon (C) cycle. Under the impact of drying and re-wetting, soils undergo complex changes of soil structure (aggregation), soil organic matter quality and microflora composition (Denef et al., 2001). Both drying and re-wetting of soils generate stress on soil microbial communities and induce significant changes in microbial C dynamics (Fierer and Schimel, 2002). Active soil microorganisms have to regulate the sudden change of osmotic potential in their cells or die following re-wetting of dry soil. The extent of microbial re-wetting stress is probably associated with the intensity of re-wetting because the amount of infiltrating water affects the change in osmotic potential of soil water (Halverson et al., 2002).

As well as direct effects on microbial activity, drying triggers indirect effects by creating hydrophobic soil surfaces even under a humid-temperate climate (Doerr et al., 2006). Hydrophobicity confers protection of SOM against microbial attack and constrains the availability of water and nutrients in soils. Hence, hydrophobicity is an important mechanism that reduces the diffusion of extracellular enzymes and nutrients and the decomposition of SOM (Goebel et al., 2007). Hydrophobicity is reversible and thus limited in time, but preferential flow of infiltrating water slows down the re-moistening of particle surfaces. Even after intensive rainfall events, a combination of hydrophobicity and preferential flow delayed the complete re-moistening of a desiccated forest floor and thereby the recovery of heterotrophic respiration in a spruce forest for some weeks (Muhr and Borken, 2009). Despite a growing number of studies on soil hydrophobicity, its relevance to the quality and turnover of SOM is not well understood.

To understand the potential of drying and rewetting for changes of SOM stocks systematic studies are required on the quality and stabilization of SOM pools in organic and mineral soil horizons. In this context, dissolved organic matter (DOM) is an important component of the soil C cycle by contributing to the build-up of SOM stock in mineral soil and by acting as C and energy source for microorganisms. Extended drought periods and subsequent rain events could modify the relevance of these functions when drying and re-wetting alter fluxes and chemical composition of DOM.

After cellulose, lignin is the main component in forest litter and represents a major input of organic matter into forest soils. Climate has an influence on the composition and contents of lignin in soils (Amelung et al., 1997; Rodionov et al., 1999). Other important SOM constituents are non-cellulose sugars which are most abundant in root exudates and non-structural plant constituents (Derrien et al., 2004).

The pentoses arabinose and xylose are characterized as plant derived sugars while the deoxy sugars rhamnose and fucose are not present in plants and are known as typical microbial markers (Glaser et al., 2000; Gross and Glaser, 2004). Plant-DNA contains deoxy ribose but not rhamnose and fucose: the latter two being analysed and used in our study as microbial markers. Advanced biodegradation of organic matter shifts the composition of neutral sugars from pentose to deoxy sugars, indicating consumption of plant derived matter and production of microbial compounds (Kaiser et al., 2004). Such changes in the ratio of plant : microbial sugars are used as a tool to trace effects of environmental disturbances on SOM degradation (Glaser et al., 2000).

Phospho lipid fatty acids (PLFA) are membrane components of all living microorganisms, but not of microbial storage products (Cifuentes and Salata, 2001). Therefore, PLFA concentration is a measure for soil microbial biomass that correlates well with methods commonly used such as substrate induced respiration (Baath and Anderson, 2003), total aldehyde content (Zelles, 1999) and chloroform-fumigation extraction in mineral

soils (Bailey et al., 2002) and forest floors (Leckie et al., 2004). PLFA characterize the current soil microbial community because they decompose rapidly after death of microorganisms (Tunlid and White, 1992). Further, there are specific PLFA for microorganisms which do not occur in plants (Frostegard et al., 1993; Cavigelli et al., 1995; Zelles, 1999). Therefore, the close relationship between microbial community and processes of SOM degradation and transformation suggests that environmental stress shifts microbial community composition (Frostegard et al., 1993; Balser and Firestone, 2005). One indicator of changes is the ratio of fungal/bacterial PLFA (fungi/sum of gram-positive bacteria + actinomycetes + gram-negative bacteria) (Fierer et al., 2003a) and the (cy17:0+19:0/ (16:1ω7c+18:1ω7c) ratio (cy/pre) which can be used as indicators of physiological or nutritional stress (Kieft et al., 1997).

Although many microorganisms face either regular or occasional drought and re-wetting stress, our knowledge about the response of microbial communities to repeated drying and re-wetting and to re-wetting intensity is sparse. PLFA profiles provide information about changes in the community of soil microorganisms or in the use of substrates following such stresses.

In this study, we use undisturbed soil columns from a Norway spruce forest; this tree species covers about one-third of the forested land in Germany. Extended summer droughts and re-wetting intensity could affect the turnover of SOM, and thus, the nutrition and growth of this economically important forest type. The aims of this study were to quantify changes in the composition of DOM, particulate SOM, and the structure of soil microbial community following repeated drying and re-wetting of a forest soil. We hypothesize i) a shift of the microbial community as indicated by an increasing ratio of fungal PLFA to bacterial PLFA and enhanced physiological/nutritional stress as indicated by an increase of the cy/pre ratio. Further, we hypothesize ii) a reduction of microbial sugars, relative accumulation of plant derived sugars and lignin phenols because of decreasing microbial activity in soils and iii) that these effects will be strongest in the mineral soil since organic horizons are better adapted

to extreme changes of moisture. To simulate different re-wetting scenarios and to test the final hypothesis iv) of different degrees of re-wetting stress on SOM quality and microbial structure the soil was re-wetted at three intensities. The accompanying measurements of DOC (Hentschel et al., 2007) and CO_2 fluxes (Muhr et al. 2008) are used to demonstrate relevant responses of the soil C budget.

2 Materials and methods

2.1 Experimental system

Undisturbed soil columns were taken from under a mountainous 135-year old Norway spruce (*Picea abies (Karst.) L.*) stand at the experimental site Coulissenhieb II, located in the Fichtelgebirge, NE Bavaria, Germany (50°8´ N, 11°52´ E) approximately 770 m above sea level. Mean annual air temperature in this area is 5.3 °C and mean annual precipitation is 1156 mm (Gerstberger et al., 2004). The soil, a Haplic Podzol, developed on weathered granite and has a sandy-loam texture (see Table 2-1 for key soil properties). The organic layer consists of Oi, Oe and Oa horizons and has a thickness of 8 to 12 cm.

Table 2-1: Mean chemical properties of soil profiles (n = 9) in the Norway spruce stand at the Fichtelgebirge (CEC_{eff} = effective cation exchange capacity; BS = base saturation).

Horizon	Depth	pH		C	N	C/N	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Al^{3+}	H^{+}	CEC_{eff}	BS
	cm	H_2O	$CaCl_2$	– % —		ratio	——— $mmol_c\ kg^{-1}$ ———							%
O_a	5	4.0	3.3	18.0	1.0	17.7	94.4	7.6	1.4	3.4	74.1	8.0	197	54
Ah	-5	4.3	3.4	7.4	0.4	19.2	60.9	3.6	1.0	1.9	66.9	7.9	145	47
Bh	-12	4.3	3.4	5.5	0.3	19.7	70.9	2.3	1.1	1.3	97.6	4.0	185	41
Bs	-18	4.6	3.7	3.4	0.2	20.5	30.1	1.1	0.9	1.0	87.8	1.3	124	27
Bw	-55	4.6	4.1	1.3	0.1	12.3	3.7	0.2	1.1	1.0	41.3	0.4	48	12
Bw/C	<-55	4.5	4.0	0.4	0.0	8.3	2.7	0.2	3.0	1.1	35.3	0.2	43	16

Two different types of soil columns were sampled using plexiglas cylinders (diameter 17.1 cm). Cylinders with a height of 15 cm were used for sampling organic layers (hereafter O columns), whereas cylinders with a height of 30 cm were used for sampling Oi, Oe, Oa, Ea,

Bhs and Bsh horizons (hereafter O+M columns). All cylinders were driven gently by hand and knife into the soil in order to reduce the disturbance of the soil structure. A total of 16 O columns and 16 O+M columns were sampled, varying in thickness and the amount of individual horizons.

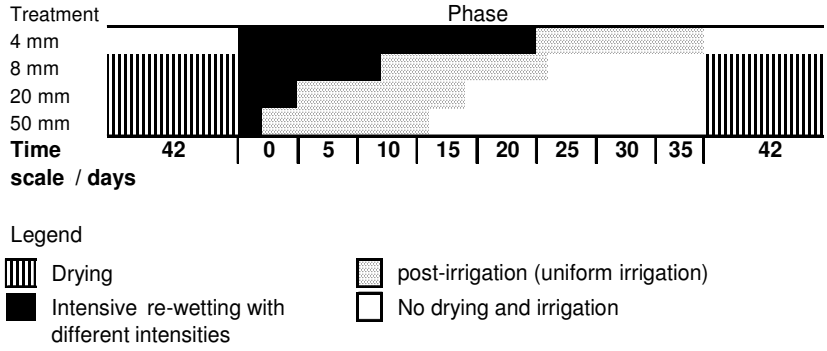

Figure 2-1: Schematic schedule of one drying and re-wetting cycle, consisting of i) drying, ii) intensive re-wetting period, during which re-wetting intensity was varying for the different treatments and iii) a post-irrigation period with uniform irrigation in all treatments. The control (4 mm of solution) was not subjected to drying and was irrigated with 4 mm d^{-1} during the intensive re-wetting and post-irrigation periods. All treatments received the same amount of water (156 mm) during each drying and re-wetting cycle.

Four different treatments with four replicates for both O and O+M columns were established to study the effect of repeated drying and re-wetting: a control (4 mm solution) and three drying and re-wetting treatments (8, 20 and 50 mm solution). The control columns were kept at 15 °C and constant soil moisture at near to field capacity throughout the experiment (Figure 2-1). All drying and re-wetting treatments were air-dried to the permanent wilting point (approximately -1500 kPa) at 20 °C for 5 – 6 weeks. Subsequently, air-dried soils were re-wetted with 8, 20 and 50 mm solution per day at 15 °C. All three treatments received 100 mm solution during the intensive rewetting period, thus the length of the intensive rewetting period varied among the treatments (12.5 days in the 8 mm, five days in the 20 mm and two days in the 50 mm treatment). After the intensive rewetting period, all treatments were uniformly irrigated at 4 mm for 14 days. The controls were permanently irrigated at 4 mm per day during the intensive rewetting and post-re-wetting periods. All control and drying and re-wetting treatments received the same amount of solution per cycle (156 mm) and which had a pH of 4.5 and similar chemical composition to that of natural

throughfall at the experimental site (Matzner et al., 2004). The same columns were subjected to three drying and re-wetting cycles under the same conditions.

2.2 Sampling

Before intensive rewetting, soil columns were placed on polyethylene plates, and sealed with a lid that allowed sampling of soil solution and measurement of CO_2 exchange between soil surface and headspace air (Hentschel et al., 2007) for the dynamics of solute concentration and fluxes including DOC (Muhr et al., 2008) and the dynamics of CO_2 fluxes during all drying and re-wetting cycles.

For the purposes of this study, soil solution from the O columns was sampled 4 times in six days intervals during the third cycle. Percolates were filtered through 0.45 µm cellulose-acetate-membrane filters (Schleicher & Schüll, Dassel, Germany) and analysed for DOC (Hentschel et al., 2007). Solutions were pooled and combined 60% of total percolate amount of each sampling date and were then freeze-dried prior to biomarker analyses (n = 4 per treatment).

After the third drying and re-wetting cycle, the O+M columns were separated into O, A and B horizons. The organic layer was cut into small pieces and mixed and the soil from mineral horizons was passed through a 2 mm sieve. Sub-sets of soil samples were dried at 40° C and ground for sugar and lignin analyses. For PLFA analyses, an aliquot of fresh, non-dried samples was kept frozen at –20 °C. Gravimetric water contents (dried at 105 °C) and the concentration of total organic C (TOC) and total N (TON) were determined of the organic layer, the A and B horizon using a Vario EL elemental analyser (Elementar, Hanau, Germany).

2.3 Analysis of lignin phenols

The sum of vanillin (V), syringyl (S) and cinamyl (C) moieties (VSC) was adopted as an

indicator of the amount of intact lignin moieties (Ziegler et al., 1986). Soil samples were oxidized with alkaline CuO to release the lignin-derived phenols vanillin, syringyl and cinamyl (modified after Hedges and Ertel, 1982). Teflon-lined bombs were loaded with an equivalent of dry soil corresponding to 25 mg TOC (10 mg C of freeze-dried material), 25 µg ethyl vanillin (Fluka Chemie, Steinheim, Germany) in 1 mL 2 M NaOH (internal standard) and heated for two hours at 170 °C on a platform shaker. After cooling to room temperature, the liquid was decanted into brown glass centrifuge tubes, the residue was washed with deionized water and centrifuged. The supernatant was acidified to pH 1.8 – 2.2 and kept at room temperature in the dark for one hour to precipitate humic acids being separated by centrifugation. An aliquot of 40 mL (100 mL of DOM samples) of the solution were added to the C_{18}–columns (Bakerbond spe^{TM} Octadecyl (C_{18}), J.T. Baker, reversed phase material, filling volume 3 mL, particle size 5 µm) and were eluted nine times with 0.5 mL ethyl acetate, being concentrated subsequently by rotary evaporation, transferred with 25 µL phenyl acetic acid (recovery standard, Fluka Chemie, Steinheim, Germany) in 1 mL methanol to 2 mL glass reaction vials and dried under nitrogen gas.

For derivatization, each CuO oxidation product was re-dissolved with 100 µL pyridine (Merck, Darmstadt, Germany) and derivatized by reaction with 200 µL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature. For quantification, derivatives were analysed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (see Schmitt et al., 2008 for further details).

2.4 Analysis of plant and microbial sugars

Plant (arabinose, xylose) and microbial (fucose, rhamnose) derived sugar extraction from soil and freeze-dried soil solution was carried out according to a modified method after Amelung et al. (1996). Ground samples containing 8 mg TOC (6 mg TOC of freeze-dried material) and an internal standard (80 µg Myo-Inositol in deionized water) were hydrolyzed with 10 mL 4

M trifluoroacetic acid flasks for four hours at 105 °C. After filtration through glass fibre filters (GF 6, Schleicher & Schüll, Dassel, Germany) the samples were dried with a rotary evaporator (-6 kPa, water temperature 45 °C). The samples were re-dissolved in 5 mL deionized water and added to stacked columns with 5 g Serdolit® PAD IV (Serva, Heidelberg, Germany) and 4 g dry Dowex® 50 WX8 cation exchange resin. The columns were washed five times with 10 mL deionized water, collected and freeze-dried. The residue containing saccharides were re-dissolved in deionized water and transferred into 3 mL glass reaction vials which were closed with parafilm and then frozen until derivatization.

Derivatization was carried out according to a modified procedure described by Gross & Glaser (2004). The frozen samples were freeze-dried and the sugars were re-dissolved in 100 µL dry pyridine (Merck, Darmstadt, Germany), containing 10 µg 3-O-methylglucose (Sigma-Aldrich, Taufkirchen, Germany) as recovery standard and in 450 µL dry pyridine containing 45 µg methylboronic acid (MBA) (Sigma-Aldrich, Taufkirchen, Germany). The samples were heated at 60 °C for one hour and diluted with 450 µL (100 µL for freeze-dried DOM) ethyl acetate after cooling. The sugar derivatives were analysed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (Schmitt et al., 2008).

2.5 Analysis of phospho lipid fatty acids (PLFA)

PLFA extraction from soil and freeze-dried DOM was carried out according to a modified method after Frostegard et al. (1991). To 5 g fresh soil (10 mg of freeze-dried material) 18 ml extraction solution was added and shaken for two hours. After centrifugation (4000 rpm for 20 minutes) the supernatant was transferred into separating funnels. Subsequently, 15 µg (10 µg in the case of freeze-dried DOM) PLFA 19:0 (internal standard, Biotrend, Cologne, Germany), 6.2 mL chloroform and 6.2 mL citrate buffer were added and shaken for 15 minutes. After separation over-night, the organic extract (lower phase) was transferred into conical flasks, dried with a rotary evaporator, re-dissolved in chloroform and separated over

glass columns filled with silica gel into neutral, glyco- and phospho- (polar) lipids (by elution with methanol). The methanol extract was dried using a rotary evaporator, transferred into 4 mL glass reaction vials with methanol and dried under a stream of N_2.

Fatty acid methyl esters (FAME) were prepared from free PLFAs using a strong acid methylation. For this aim, the PLFA were re-dissolved in 0.5 mL 0.5 M NaOH in methanol, and heated at 100 °C for 10 minutes. After the addition of 0.75 mL boron-trifluoride in methanol (13 – 15% concentration of BF_3; Fluka, Seelze, Germany) the samples were heated at 80 °C for 15 minutes. After addition of 0.5 mL saturated NaCl solution, the samples were shaken three times for 30 seconds with 1 mL hexane. The hexane phase was pipetted into another reaction vial and dried under a stream of nitrogen. The PLFAs were re-dissolved in 10 µL (5 µL for DOM) 13:0 FAME (Sigma-Aldrich, Taufkirchen, Germany) in toluene as recovery standard and 490 µL (95 µL in the case of DOM) toluene and transferred into GC auto-sampler vials. The derivates were analysed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (Schmitt et al., 2008).

Terminal branched saturated PLFAs (a15:0, i15:0, i16:0, i17:0, a17:0) were considered as makers for gram-positive bacteria and mid-chain branched saturated PLFAs (10Me16:0, 10Me17:0, 10Me18:0) were associated with actinomycetes. Typical for gram-negative bacteria are mono-unsaturated fatty acids (16:1ω7c, 18:1ω7c) and cyclopropyl saturated PLFAs (cy17:0, cy19:0). Short or odd-chain saturated PLFAs (14:0, 15:0, 16:0, 17:0, and 18:0) were considered as non-specific bacterial makers and are present in all microbial organisms groups. Typical markers for fungi are PLFA 18:2ω6,9, 18:1ω9c and 16:1ω5c (Stahl and Klug, 1996; Zelles, 1999; Myers et al., 2001; Ruess et al., 2002; DeForest et al., 2004; Waldrop et al., 2004; McMahon et al., 2005).

2.6 Statistics

Statistical analyses were carried out using STATISTICA 5.0. Differences between different

drying and re-wetting treatments were evaluated using a one-way ANOVA followed by the Tuckey-Honest post-hoc test.

3 Results and discussion

3.1 Effect of drying and re-wetting on DOM quality

Drying and different re-wetting intensities did not affect the concentration of VSC lignin, plant and microbial sugars, and PLFAs in DOC leached from the O columns (Figure 2-2a-e). All these components made only small contributions (< 3%) to the overall DOC flux (Table 2-2), indicating that DOC is dominated by other constituents. The largest fractions were sugars followed by VSC lignin and PLFAs.

Table 2-2: Mean cumulative fluxes (± SE) (g m^{-2}) of lignin, total sugars and PLFA in the soil solution from the O columns of the control (4 mm), 8, 20 and 50 mm treatments with added solution during the third drying and re-wetting cycle. Cumulative DOC and CO_2 fluxes (g C m^{-2}) of three drying and re-wetting cycles were measured by Hentschel *et al.* (2007) and Muhr *et al.* (2008), respectively.

Constituent	4 mm	8mm	20mm	50mm
Lignin (VSC)	0.13 ± 0.04	0.26 ± 0.05	0.16 ± 0.01	0.19 ± 0.03
Total sugar	0.58 ± 0.16	0.59 ± 0.19	0.61 ± 0.06	0.55 ± 0.35
PLFA	0.06 ± 0.00	0.08 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
DOC	19 ± 7.5	24 ± 4.9	21 ± 0.9	26 ± 2.2
CO2-C	101 ± 17	94 ± 14	98 ± 3.5	95 ± 11

Although the treatment had no significant effect on sugar concentrations (Figure 2-2b and 2-2c), the ratio of plant to microbial sugars was about two times greater in the treatments compared with the control (Figure 2-2d). This shift might indicate microbial stress by drying and re-wetting as less plant sugar was transformed to microbial sugars. The fact that re-wetting intensity had no effect on the sugar ratio does not exclude the possibility that re-wetting *per se* causes no stress for soil microorganisms. However, changes in the quality of DOC are not only a result of microbial activity, but possibly also a result of sorption and desorption processes (Müller et al., 2009). Based on spectroscopic properties, the quality of

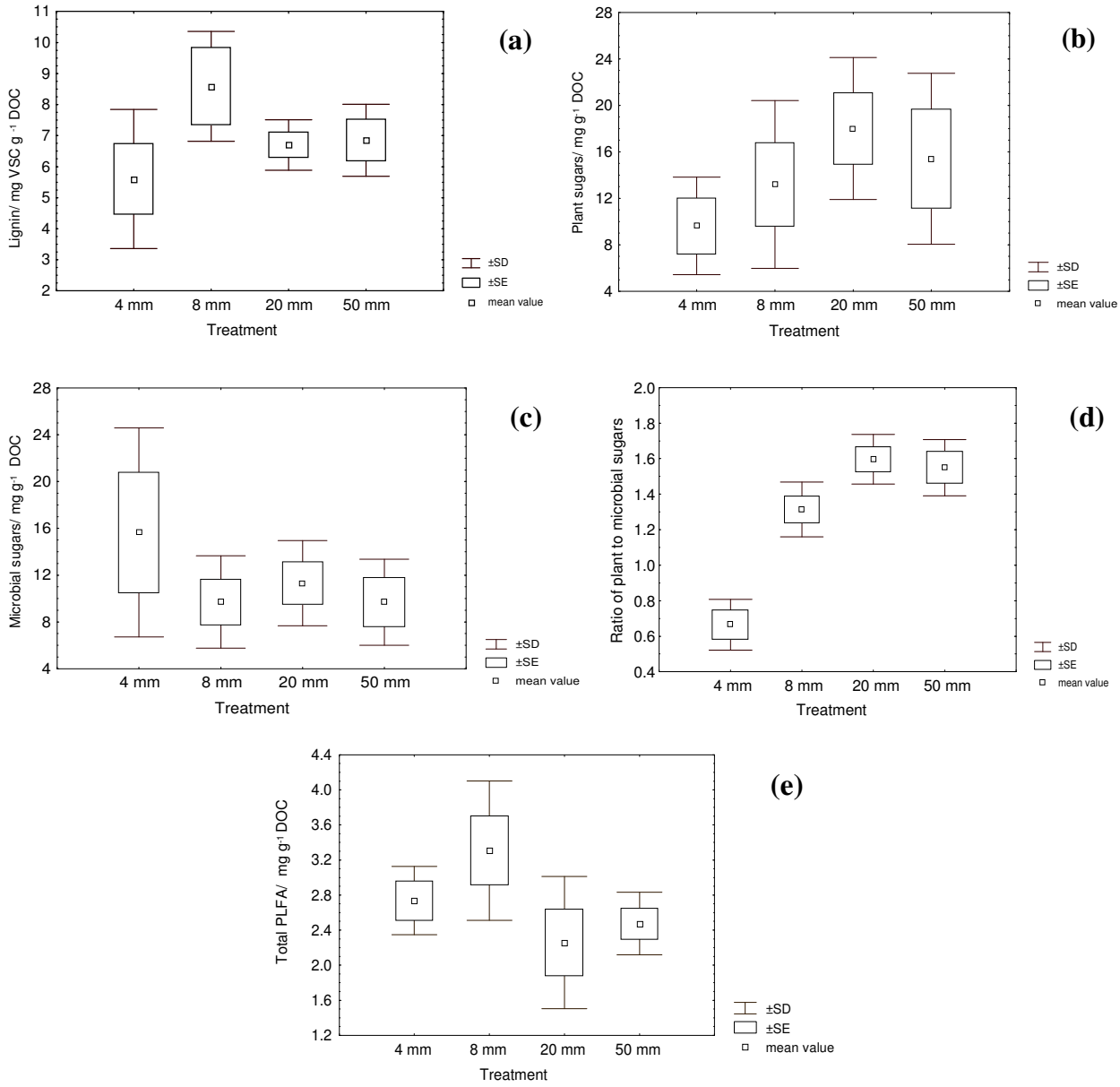

Figure 2-2: Concentration of (a) lignin phenols (sum of VSC), (b) plant sugars, (c) microbial sugars, (d) ratio of plant to microbial sugars, and (e) sum of PLFAs (n = 21) in soil solution from the O horizons of the control (4 mm), 8, 20 and 50 mm treatments with added solution of the third drying and wetting cycle.

DOC changed from less to more humified substrates within each drying-wetting cycle and from the first to the third cycle (Hentschel et al., 2007). Enhanced desorption and leaching of less decomposable substrates and reduced microbial DOC production could have contributed to the shift in the ratio of plant to microbial sugars in the third cycle. Slightly elevated concentrations of VSC lignin indicate either an increased release of VSC lignin or a decreased mineralization of VSC lignin in soil solution of the treatments (Figure 2-2a, Table 2-2). In fact, the concentration of DOC was always greater in the treatments than in the

control; there was, however, usually no effect on cumulative DOC fluxes because of smaller water-flow rates (Hentschel et al., 2007). CO_2 losses by heterotrophic respiration exceeded DOC losses considerably (Table 2-2) and this indicates there was no general stress for heterotrophic microorganisms in the O horizons during re-wetting (Muhr et al., 2008). Hence, the shift in the ratio of plant to microbial sugars is not linked with SOM mineralization.

3.2 Effect of drying and re-wetting on soil organic carbon (SOC) quality

Table 2-3: Mean stocks (± SE) (g m^{-2}) of SOC, lignin, total sugars and PLFA in the O, A and B horizons (O+M columns) of the control (4 mm), 8, 20 and 50 mm treatments with added solution after three drying and re-wetting cycles. Cumulative DOC and CO_2 fluxes (g C m^{-2}) of three drying and re-wetting cycles were measured by Hentschel *et al.* (2007) and Muhr *et al.* (2008), respectively.

Horizon	Constituent	4 mm	8mm	20mm	50mm
O	SOC	4910 ± 755	3893 ± 384	3040 ± 487	3861 ± 494
	Lignin (VSC)	333 ± 61	296 ± 34	232 ± 45	184 ± 19
	Total sugar	1644 ± 339	993 ± 123	661 ± 215	543 ± 28
	PLFA	2.4 ± 0.3	3.3 ± 1.0	4.6 ± 2.3	6.3 ± 2.5
A	SOC	1970 ± 206	1283 ± 81	1744 ± 204	1524 ± 471
	Lignin (VSC)	87 ± 8.9	59 ± 3.0	58 ± 7.1	39 ± 13
	Total sugar	125 ± 27	33 ± 16	38 ± 15	20 ± 4
	PLFA	5.5 ± 1.5	5.2 ± 1.4	4.0 ± 1.3	6.6 ± 2.6
B	SOC	4596 ± 670	4666 ± 273	6191 ± 269	5136 ± 339
	Lignin (VSC)	89 ± 30	132 ± 19	100 ± 6.8	102 ± 11
	Total sugar	195 ± 49	92 ± 21	92 ± 36	60 ± 20
	PLFA	23 ± 10	45 ± 13	35 ± 20	85 ± 13
	DOC	4.9 ± 0.4	7.6 ± 2.2	6.3 ± 0.88	6.7 ± 1.8
	CO_2	151 ± 16	117 ± 10	110 ± 10	112 ± 19

The investigated C pools in the O horizon generally increased in the order PLFA < VSC lignin < total sugars (Table 2-3). A distinct pattern was found for the mineral soil where, except for the control, the amounts of VSC lignin were greater than the amounts of total sugars. Differences in C pools among the treatments can be attributed to differences in the thickness of the O, A and B horizons and partly to the drying and re-wetting cycles. In the following sections we focus on contents of VSC lignin, sugars and PLFAs, and use DOC and

CO_2 fluxes as supportive information for discussion of observed treatment effects.

3.2.1 Lignin phenols

The contents of extractable lignin phenols (VSC) varied between 16 and 76 mg g^{-1} SOC and decreased in the order O horizon > A horizon > B horizon in all treatments (Figure 2-3). The contents and vertical distribution of VSC lignin are in agreement with other studies in forest soils (Glaser et al., 2000; Spielvogel et al., 2007).

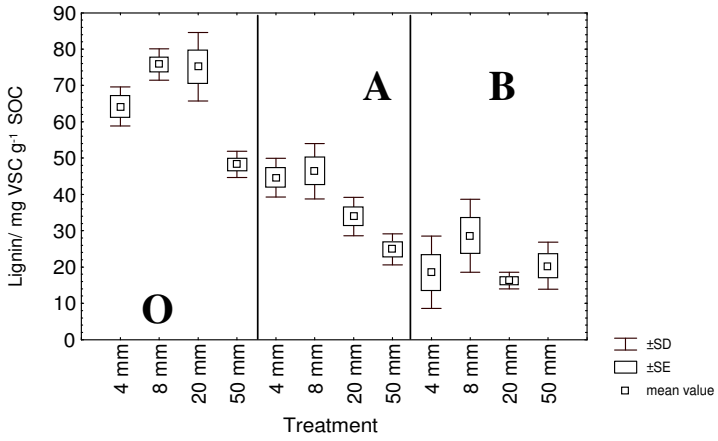

Figure 2-3: Content of lignin phenols (sum of VSC) in the O, A and B horizons of the control (4 mm), 8, 20 and 50 mm treatments with added solution after the third drying and wetting cycle.

Contents of VSC lignin exhibited no systematic and consistent changes in the O, A and B horizons following drying and re-wetting (Figure 2-3). Only the VSC lignin contents of the 50 mm treatment significantly decreased in both the O and A horizons. These losses cannot be explained by increased DOC output or by increased CO_2 fluxes. In contrast, we expected greater contents of VSC lignin because of decreasing C mineralization. One reason for less VSC lignin in the O and A horizons could be a chemical transformation of lignin derivates by the combination of drying and 50 mm re-wetting intensity. Further, vertical translocation of metal-organic complexes containing lignin components could have contributed to the loss of VSC lignin. Elevated DOC fluxes in the O horizon (Table 2-2) and rapid water movement along macropores following re-wetting of dry soil increase the mobility and translocation of DOC into the B horizon. Both mechanisms (transformation and translocation) would reduce the amount of extractable lignin phenols in the O and A horizons.

3.2.2 Plant and microbial sugars

The contents of plant and microbial sugars decreased in the order O horizon >> A horizon > B horizon (Figures 2-4 and 2-5) in line with other data (Rumpel et al., 2002). The contents of plant sugars ranged between 6.9 and 268 mg g^{-1} SOC and were consistently greater than those of microbial sugars (4.4 and 65.4 mg g^{-1} SOC) although the ratio of microbial to plant sugars increased from the O to the B horizon. While sugar contents of the A and B horizons corresponded well with results from other studies, contents of the organic horizons were greater than in other forest soils (Glaser et al., 2000; Spielvogel et al., 2007). Drying and re-wetting reduced the mean contents of plant and microbial sugars in all soil horizons (Figures 2-4 and 2-5). The 8 mm treatment was generally less effective and not significantly different to the control when compared with the 20 and 50 mm re-wetting intensity. We expected a relative accumulation of plant sugars and a small decline in microbial sugars as a result of decreasing microbial activity during the drying periods. Carbon mineralization did not recover even during the re-wetting periods (Muhr et al., 2008), suggesting that less plant sugar was consumed or transformed to microbial sugars. Thus, the observed losses of plant sugars are counter to the decrease in CO_2 fluxes and losses by DOC leaching are too small to explain the differences (Tables 2-2 and 2-3). We can only speculate about the fate of plant sugars in the 8, 20 and 50 mm treatments as similar results have not been reported in the literature so far. It is possible that a considerable portion of plant sugars was chemically transformed into other compounds by drying and re-wetting. Analyses of sugar derivatives by capillary gas chromatography are specific, and thus, minor modifications of the molecular structure of sugars would under-estimate the 'true' sugar content. We cannot exclude the possibility that similar transformation processes affected microbial sugars although we expected decreasing or constant microbial sugar contents in our experiment. The analytical method itself is unlikely to be responsible for the observed changes in sugar contents. Extraction of ground

samples with trifluoroacetic acid for four hours at 105 °C should release carbohydrate monomers from non-cellulose sugars by direct contact of extraction solution with SOM. Even physically protected sugars in soil aggregates will probably not resist this treatment. According to Spielvogel et al. (2007), the method is able to quantify organo-mineral stabilized sugars in soils. If mineral surfaces had stabilized plant sugars during drying and re-wetting, such a mechanism would not explain the decline of sugar contents in the organic layer.

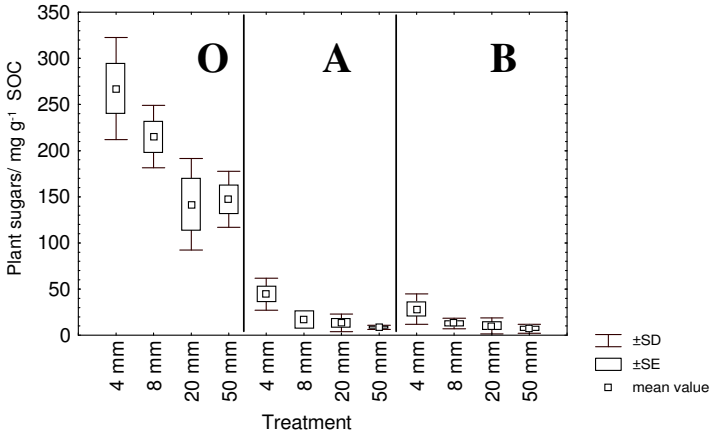

Figure 2-4: Content of plant sugars in the O, A and B horizons of the control (4 mm), 8, 20 and 50 mm treatments with added solution after the third drying and wetting cycle.

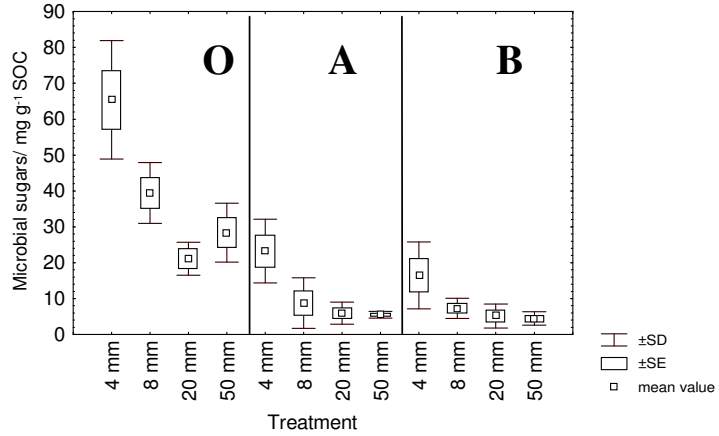

Figure 2-5: Content of microbial sugars in the O, A and B horizons of the control (4 mm), 8, 20 and 50 mm treatments with added solution after the third drying and wetting cycle.

It should be noted that all soil samples from the control and the treatments were consistently air-dried at 40 °C before extraction. This procedure did not remove the differences between the control and the drying and re-wetting treatments, suggesting that repeated drying and re-wetting triggered chemical transformation of plant sugars. Hydrophobicity of SOM is theoretically a relevant mechanism because of spatial re-

arrangement of hydrophilic, hydrophobic and amphiphilic molecules during soil drying. A re-arrangement of sugars and other molecules might be a requisite for chemical transformation of sugars. It remains unclear whether chemical modification of plant sugars has an impact on microbial accessibility and mineralization of the sugar pool as well as the influence on the SOC stock under field conditions in the long run.

The fact that the amount of extractable plant sugars decreased in our experiment does not exclude physical protection and decreasing microbial accessibility during the drying and in part during the re-wetting periods. Although not measured, hydrophobicity had an apparently severe effect in our experiment because the final water contents were smaller in the treatments than in the control columns. As well as hydrophobicity, the incomplete re-moistening of the soil columns can be attributed to rapid flow of added water along preferential flow paths, preventing homogenous distribution of water on particle surfaces. Our observation is consistent with the study by Bogner et al. (2009) who reported preferential flow in the top soil of the same spruce stand at wetter conditions. In contrast to many other laboratory studies, we used intact soil columns where hydrophobicity and preferential flow should have had a similar influence on sugar pools to that under field conditions.

Provided that the analytical method did not affect the ratio of plant to microbial sugars, drying and re-wetting reduced the portion of microbial sugars in the organic layer data not shown). This shift indicates less production of microbial sugars because of decreasing microbial activity during drying and re-wetting. In a review, Borken and Matzner (2009) concluded that elevated C mineralization following re-wetting of dry soil cannot compensate for reduced C mineralization during drought periods. This is particularly relevant for organic horizons where drying and re-wetting is a frequent event. Both, drying and rewetting induce stress for active microorganisms that may cause cell lysis and death of some microorganisms under extreme conditions (Clein and Schimel, 1994).

3.2.3 Phospho lipid fatty acids (PLFA)

The total stock of PLFA as a relative measure for soil microbial biomass increased in the order O horizon < A horizon << B horizon (Table 2-3). The contents of PLFA ranged between 0.4 and 0.9 mg g^{-1} SOC in the O horizon and between 1.4 and 18.6 mg g^{-1} SOC in the mineral horizons (not shown). Similar PLFA contents were reported for *Pinus sylvestris*, *Picea abies* and *Betula pendula* forest soils in Finland (Priha et al., 2001). In contrast to the vertical pattern of PLFA, C mineralization was greater in the O horizon compared with the mineral soil (Tables 2-2 and 2-3), indicating a less active soil microbial biomass in the mineral soil. In agreement with Fierer et al., (2003b) the ratio of fungi to bacteria decreased from the O horizon (0.25 – 0.32) to the A horizon (0.14 – 0.23) and the B horizon (0.15 – 0.21) (Table 2-4). This shift indicates that fungi are better adapted to the environmental conditions in the O horizon than bacteria and *vice versa*. Comparing the control treatment with the 8, 20 and 50 mm treatments, such an adaptation corresponds well with the small decrease in cumulative C mineralization in the O horizon (-3 to -7 g C m^{-2}, Table 2-2) and the strong decrease in the mineral soil (-41 to -34 g C m^{-2}, Table 2-3).

Despite the strong decrease in C mineralization, PLFA stocks (Table 2-3) as well as PLFA concentrations (data not shown) in the mineral soil were barely affected by repeated drying and re-wetting at different intensities. (Wilkinson et al., 2002) also found no significant changes in microbial PLFA concentrations of spruce and pine litter induced by drought stress. In contrast, soil drying caused on average a decrease in the size of microbial biomass with respect to moist samples (Mondini et al., 2002). Microbial populations might respond differently to drought stress depending on the composition of soil microorganisms and the history of drying and re-wetting. In addition to the variation of microbial communities in different soils and soil horizons, stress conditions such as the duration, intensity and frequency of drying and re-wetting vary from study to study.

Table 2-4: Contributions ± SE of individual PLFAs to sum of PLFAs (%) in the O, A and B horizons of the control (4 mm), 8, 20 and 50-mm treatments with added solution after the third drying-rewetting cycle (n = 4).

		Organic layer				A horizon				B horizon			
		4mm	8mm	20mm	50mm	4mm	8mm	20mm	50mm	4mm	8mm	20mm	50mm
Common	14:0	2.5 ± 0.1	2.9 ± 0.3	3.0 ± 0.1	3.5 ± 0.2	2.7 ± 0.2	3.6 ± 0.5	3.2 ± 0.2	3.0 ± 0.1	2.1 ± 0.1	2.4 ± 0.2	2.7 ± 0.3	2.3 ± 0.1
	15:0	1.7 ± 0.1	2.2 ± 0.3	2.0 ± 0.0	1.3 ± 0.2	1.1 ± 0.2	1.6 ± 0.6	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.0
	16:0	20.1 ± 1.3	23.3 ± 1.2	22.0 ± 0.8	18.7 ± 0.8	11.1 ± 1.1	12.1 ± 0.6	12.5 ± 0.5	11.4 ± 0.4	15.9 ± 0.5	15.6 ± 1.7	15.3 ± 1.1	14.4 ± 0.2
	17:0	1.0 ± 0.1	1.2 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	2.2 ± 0.3	1.9 ± 0.3	2.1 ± 0.2	1.6 ± 0.4
	18:0	8.7 ± 0.9	9.7 ± 2.5	4.6 ± 0.6	4.2 ± 0.3	4.3 ± 1.3	4.1 ± 0.6	4.2 ± 0.8	3.6 ± 0.8	7.4 ± 0.7	4.5 ± 0.2	6.7 ± 1.5	4.8 ± 0.3
Gram-positive	i15:0	8.6 ± 1.1	8.5 ± 1.0	9.9 ± 0.3	8.8 ± 0.5	9.9 ± 0.9	11.4 ± 1.0	10.5 ± 0.6	9.3 ± 0.5	11.8 ± 0.2	10.2 ± 0.5	8.6 ± 0.7	8.8 ± 0.4
	a15:0	3.0 ± 0.1	2.5 ± 0.1	2.5 ± 0.2	1.9 ± 0.2	2.6 ± 0.1	2.5 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	2.4 ± 0.4	2.1 ± 0.2	2.0 ± 0.1	2.3 ± 0.1
	i16:0	8.5 ± 1.7	9.3 ± 1.7	9.8 ± 0.7	5.7 ± 0.3	6.6 ± 0.8	6.6 ± 0.6	7.7 ± 0.7	6.6 ± 1.0	5.9 ± 0.8	4.8 ± 0.5	4.7 ± 0.3	4.0 ± 0.5
	i17:0	5.5 ± 1.4	4.1 ± 1.4	2.6 ± 0.8	1.5 ± 0.2	1.6 ± 0.5	2.1 ± 0.7	1.9 ± 0.6	1.0 ± 0.1	3.2 ± 0.2	2.6 ± 0.0	2.7 ± 0.2	2.7 ± 0.2
	a17:0	2.2 ± 0.1	2.4 ± 0.1	2.6 ± 0.4	2.0 ± 0.1	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
Actinomycetes	10Me16:0	7.5 ± 1.2	8.0 ± 1.0	8.0 ± 0.3	7.0 ± 0.8	7.0 ± 0.4	8.3 ± 0.8	7.6 ± 0.4	7.0 ± 0.3	6.4 ± 0.2	7.2 ± 0.7	6.9 ± 0.8	6.3 ± 0.3
	10Me17:0	1.2 ± 0.2	1.3 ± 0.2	1.8 ± 0.2	1.4 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
	10Me18:0	2.7 ± 0.4	2.3 ± 0.5	1.5 ± 0.3	1.3 ± 0.1	1.8 ± 0.1	3.1 ± 0.8	2.4 ± 0.3	1.4 ± 0.2	3.1 ± 0.2	2.3 ± 0.3	1.9 ± 0.2	2.1 ± 0.2
Gram-negative	16:1ω7c	2.8 ± 0.5	2.4 ± 0.6	3.6 ± 0.3	3.6 ± 0.2	4.0 ± 0.7	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.1	3.3 ± 0.3	3.9 ± 0.5	3.1 ± 0.5	2.3 ± 0.2
	18:1ω7c	3.1 ± 0.5	2.2 ± 0.5	2.9 ± 0.2	4.2 ± 0.4	4.7 ± 1.0	2.3 ± 0.4	3.5 ± 0.7	3.7 ± 0.6	3.8 ± 0.6	4.9 ± 0.8	4.2 ± 0.6	5.5 ± 0.7
	cy17:0	0.6 ± 0.2	0.7 ± 0.3	1.4 ± 0.1	2.0 ± 0.2	1.2 ± 0.2	1.8 ± 0.4	1.8 ± 0.2	1.9 ± 0.2	1.0 ± 0.2	1.7 ± 0.3	1.5 ± 0.2	1.3 ± 0.3
	cy19:0	3.4 ± 1.0	3.0 ± 1.7	5.8 ± 0.4	12.4 ± 1.4	12.2 ± 1.5	12.1 ± 1.2	15.7 ± 2.7	19.1 ± 1.5	10.5 ± 0.6	14.3 ± 0.3	15.3 ± 0.6	16.6 ± 1.5
Fungi	18:2ω6,9	3.3 ± 0.5	2.7 ± 0.4	3.2 ± 0.2	3.6 ± 0.4	1.8 ± 0.3	1.6 ± 0.1	1.6 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
	18:1ω9c	11.1 ± 0.9	9.6 ± 1.0	9.1 ± 0.6	10.1 ± 0.4	7.2 ± 0.2	9.5 ± 0.2	7.4 ± 0.7	6.6 ± 0.4	9.3 ± 0.6	8.8 ± 1.6	7.6 ± 0.7	7.8 ± 0.3
VAM	16:1ω5c	1.0 ± 0.1	0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.0	1.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	0.8 ± 0.1
Protozoa	20:4ω6	1.3 ± 0.4	0.9 ± 0.3	1.6 ± 0.3	4.6 ± 0.1	19.1 ± 5.5	13.5 ± 2.6	8.7 ± 1.9	12.9 ± 2.6	6.7 ± 2.2	11.7 ± 1.8	13.3 ± 2.3	12.4 ± 1.3
Fungi/bacteria ratio		0.30 ± 0.0	0.27 ± 0.1	0.23 ± 0.0	0.27 ± 0.0	0.18 ± 0.0	0.22 ± 0.0	0.15 ± 0.0	0.14 ± 0.0	0.20 ± 0.0	0.20 ± 0.1	0.18 ± 0.0	0.16 ± 0.0

3.3 Effect of drying and re-wetting on structure of soil microbial community

An indicator of physiological or nutritional stress of gram-negative bacteria is the ratio of the sum of cyclopropyl PLFAs to the sum of their monoenoic precursors (cy17:0+cy19:0)/(16:1w7c+18:1w7c); abbreviated as cy/pre (Kieft et al., 1997). Even if the total microbial population was barely affected by drying and re-wetting, the increasing cy/pre ratio (Figure 2-6) demonstrated that metabolism of gram-negative bacteria changed at least at the most intensive re-wetting treatment producing more cyclic fatty acids and/or less unsaturated fatty acids. The greatest stress appeared in the A horizon where all treatments had larger cy/pre ratios. The B horizon was generally not significantly affected by drying and re-wetting.

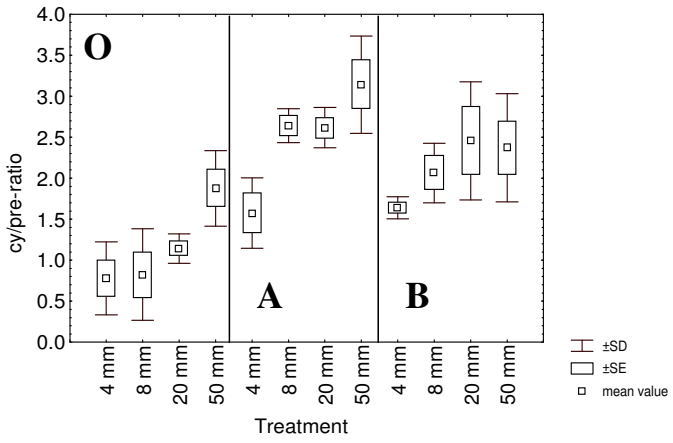

Figure 2-6: Ratio of cy/pre [(cy17:0+cy19:0)/(16:1ω7c+18:1ω7c)] in the O, A, and B horizons of the control (4 mm), 8, 20 and 50 mm treatments with solution after the third drying and wetting cycle.

If one regards the individual PLFA and sets this in relation to the total sum of PLFA, some significant changes occurred with respect to re-wetting intensity (Table 2-4). In the O and B horizons, the gram-positive markers i15:0, a15:0, i17:0 and the actinomycetes marker 10Me18:0 significantly ($p < 0.05$) decreased while the gram-negative markers 18:1ω7c, cy17:0 and cy19:0, as well as the protozoan marker 20:4ω6, significantly increased ($p < 0.05$) with re-wetting intensity. The response of these individual markers to drying and re-wetting was less pronounced in the A horizon. Microbial communities did not respond in the same way in different soil horizons because some communities gained and others lost by the

treatments. Variations in the soil environment resulted in the differentiation of spatially defined bacterial communities with some being more tolerant to drought stress than others (Wilkinson et al., 2002). Rapid changes in soil water potential may also select for bacteria and fungi which have thicker, more rigid cell walls and compatible solutes that enhance osmo-regulatorly capabilities (Schimel et al., 1999). Our results do not support a shift from bacterial to fungal biomass as the fungi/bacteria ratio was usually smaller in the 8, 20 and 50 mm treatments compared with the control (Table 2-4). However, PLFA pattern in the soil horizons were investigated approximately five weeks after the third drying and re-wetting event. During this period recovery of total bacteria population was possibly faster than recovery of fungal growth. In a grassland desert, the response of bacteria and fungi to rain events was affected by temperature (Bell et al., 2008). The response of fungi was stronger during cooler periods whereas the response of bacteria was barely affected by seasonal variation of temperature. The uniform and relatively high temperature in our laboratory experiment might have promoted bacterial more than fungal growth during intensive re-wetting and post irrigation.

4 Conclusions

Our drying and re-wetting experiment revealed few systematic changes of SOM pools while C mineralization was consistently reduced in all treatments. The decrease of extractable plant sugars in the organic horizons and mineral soil do not concur with the reduction of C mineralization. Physical stabilization of sugars arising from drying and re-wetting induced changes in soil structure can be ruled out as an explanation because of the applied extraction method. We postulate chemical transformation of sugar molecules following drying and re-wetting. Further studies are required to understand the mechanisms of these modifications and to examine whether these changes affect only the extraction of sugars or also the stabilization of the SOM pool. Hydrophobicity of SOM is possibly an important mechanism that persists

even some weeks after re-wetting thereby reducing the mineralization of SOM pools. The increasing cy/pre-ratio [(cy17:0+cy19:0)/(16:1ω7c+18:1ω7c] with re-wetting intensity indicates physiological or nutritional stress for gram-negative bacteria in the spruce forest soil. Considering the pattern in C mineralization we conclude that microbial communities of organic horizons are better adapted to drying and rewetting than those of the mineral soil. Changes in the bacterial community structure in the mineral soil by severe drought stress could have the potential to affect the C-cycle of spruce forests. The intensity of re-wetting has little effect on the activity and community of microorganisms in forest soils because heterogeneous water infiltration along preferential flow paths and hydrophobicity possibly caused similar, incomplete pattern of water availability in the soil.

Acknowledgements

This study was funded by the German Research Foundation within the Research Group 562 "Soil processes under extreme meteorological boundary conditions" (BO 1741/3-2).

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Study 3

Organic matter dynamics in a temperate forest as influenced by soil frost

Andrea Schmitt [(a*)] and Bruno Glaser [(a,b)]

[(a)] Soil Physics Department, University of Bayreuth, D-95440 Bayreuth, Germany

[(b)] Current address: Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany.

[*] Corresponding author: Andrea-G.Schmitt@gmx.de

Journal of Plant Nutrition and Soil Science

In press 2011

Abstract

In the future, climate models predict an increase in global surface temperature and during winter a changing of precipitation from less snowfall to more raining. Without protective snow cover, freezing can be more intensive and can enter noticeably deeper into the soil with effects on C cycling and soil organic matter (SOM) dynamics. We removed the natural snow cover in a Norway spruce forest in the Fichtelgebirge mountains during winter from late December 2005 until middle of February 2006 on three replicate plots. Hence we induced soil frost to 15 cm depth (in a depth of 5 cm below surface up to -5 °C) from January to April 2006, while the snow-covered control plots never reached temperatures below 0 °C. SOM quantity and quality was followed by total organic C and biomarker analysis. While soil frost did not influence total organic carbon and lignin concentrations, the decomposition of vanillyl monomers $(Ac/Ad)_V$ and the microbial sugars concentrations decreased at the end of the frost period, these results confirm reduced SOM mineralisation under frost. Soil microbial biomass was not affected by the frost event or recovered more quickly than the accumulation of microbial residues such as microbial sugars directly after the experiment. However, in the subsequent autumn, soil microbial biomass was significantly higher at the snow removal (SR) treatments compared to the control despite lower CO_2 respiration. In addition, the water stress indicator [PLFA (cy17:0+cy19:0)/ (16:1ω7c+18:1ω7c)] increased. These results suggest that soil microbial respiration and therefore the activity was not closely related to soil microbial biomass but more strongly controlled by substrate availability and quality. The PLFA pattern indicates that fungi are more susceptible to soil frost than bacteria.

Keywords: climate change, extreme weather events, freeze-thawing, biomarker, lignin, sugar, microbial community, phospholipid fatty acid

1 Introduction

Climate models predict an increase in global surface temperature and change of precipitation intensity during this century (IPCC, 2007). Henry (2007) reported increased annual soil freeze-thaw cycles in both warm and dry winters for Canada. However, the annual soil freezing days declined with increasing mean winter air temperature despite decreases in snow cover thickness, and reduced precipitation only increased annual soil freezing days in the warmest sites. Also for Europe, climate models prognose an increase in Earth surface temperature. The temperature will increase between 0.6 und 1.5 °C depending on the region and an increase of precipitation is observed during winter (Gerstengarbe and Werner, 2008). A lack of snow cover or a late snowfall in winter results in soil freezing being deeper and of longer duration than when the snow pack is established in early winter (Fitzhugh et al., 2001). Therefore, without protective snow cover, freezing can be more intensive and can enter noticeably deeper into the soil. Alternatively, more frequent thawing periods during winter may occur.

Numerous studies exist on the effects of frost on C and N cycles. The C turnover affected by frost was well examined until now, but the attention was usually more directed towards the effects of freezing-thawing on dissolved organic carbon (DOC) (Kawahigashi et al., 2006; Hentschel et al., 2009) respiration (CO_2) (Muhr et al., 2009; Goldberg et al., 2010) or total soil organic carbon (TOC, SOC) (Neilson et al., 2001; Matzner and Borken, 2008). However, as soil organic matter (SOM) consists of ecologically different pools such as labile (active), intermediate (slow) and stable (passive) pools, these different SOM pools may react differently to soil freeze / thaw cycles. The active or labile SOM pool comprises sugars, organic acids and carbon of death soil microbial biomass with a mean residence time (MRT) of days to months (Cochran et al., 2007). The intermediate or slow SOM pool comprises structural plant residues with a MRT of 25 – 50 years and the stable SOM pool consists of lignin and chemically stabilized C with MRT of 1000 – 1500 years (Cochran et al., 2007).

Lignin is a main component in forest litter and represents a major input of organic matter into forest soils (Ziegler et al., 1986). Lignin compounds are phenolic polymers consisting of vanillin (V), syringyl (S), and cinamyl (C) moieties found in the cell walls of all vascular plants (Hedges and Ertel, 1982). The sum of V+S+C (VSC) after alkaline CuO oxidation was adopted as an indicator of the amount of intact lignin moieties. However, a quantification of lignin in soil is not possible with this method, neither with other known analytical methods (Ziegler et al., 1986), nor as a result of the complex structure of lignin in combination with other organic compounds such as cellulose. Nevertheless, the alkaline CuO oxidation method releasing phenols from reactive sites of the lignin macromolecule is a relative measure for the lignin content in soils (Amelung et al., 1999). The gymnosperm lignin consists of 80 % coniferyl alcohol-derived units (vanillyl phenols) (Ziegler et al., 1986) and the lignin molecule is typically altered during decomposition by white rot fungi that oxidize aldehyde units and produce acid units (Koegel, 1986). Therefore, the state of degradation can be recognized by the ratio of the oxidized derivatives versus the corresponding aldehyde $(Ac/Ad)_V$ of the vanillyl monomers (Koegel-Knabner, 2000).

Non-cellulosic sugars are important SOM compounds being mostly abundant in root exudates and non-structural plant constituents (Derrien et al., 2004). While the pentoses arabinose and xylose are mainly plant-derived, hexoses and deoxy sugars such as fucose and rhamnose are of microbial origin (Gross and Glaser, 2004). Advanced biodegradation of SOM shifts the composition of neutral sugars from pentoses to deoxy sugars indicating consumption of plant-derived organic matter and production of microbial compounds (Murayama, 1984; Kaiser et al., 2004). The ratio of arabinose + xylose to fucose + rhamnose is a tool to trace effects of environmental changes on SOM degradation (Amelung et al., 1999; Glaser et al., 2000).

Phospholipid fatty acids (PLFA) are membrane components of all living micro organisms but not of microbial storage products (Zelles et al., 1992; Zelles et al., 1994).

Therefore, PLFA content is a measure for soil microbial biomass correlating with commonly used methods such as substrate-introduced respiration (Zelles et al., 1994; Zelles, 1999), total aldehyde content (e.g. Zelles, 1999) chloroform-fumigation extraction in mineral soils (e.g. Bailey et al., 2002) and in forest floors (Leckie et al., 2004). After death of the micro organisms, PLFA are rapidly decomposed, therefore the close relationship between microbial community composition and soil processes suggests that changes in the ecosystem through environmental stress such as freezing/thawing events shift microbial community composition (Frostegard et al., 1993). The ratio of fungal/bacterial PLFA (fungi/sum of gram-positive bacteria + actinomycetes + gram-negative bacteria) can be used as indicator for nutritional stress (Fierer and Schimel, 2003). A further indicator of physiological or nutritional stress in bacterial communities is the increasing ratio of the sum of cyclopropyl PLFA to the sum of their monoenoic precursors (cy17:0+cy19:0)/(16:1ω7c+18:1ω7c; abbreviated as cy/pre ratio) (Kieft et al., 1997).

Groffmann et al. (2001) already conducted a snow removal experiment in the northern hardwood forest ecosystem at the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, U.S.A during the winters 1997/1998 and 1998/1999. The authors found that soil frost had implications for changes in soil biogeochemical processes, like physical disruption of soil aggregates or increased N and P losses. Physical disruption can increase fine root mortality, reduce plant N uptake and reduce competition for inorganic N, allowing soil NO_3^- levels to increase even with no increase in net mineralization or nitrification. Individual components of SOM were not examined regarding frost effects, although biomarkers provide information about changes of SOM quality, the community of soil microorganisms or in the use of substrates after environmental disturbance (Sulkava and Huhta, 2003; Borjesson et al., 2004). However, there is little information regarding the changes of the biomarkers on frost events. Therefore, the focus of this work was on the characterization of different SOC pools by means of the biomarkers lignin (stable pool),

plant- and microbial-derived sugars (labile pool) and phospholipid fatty acids (PLFA) as indicators for soil microbial groups.

A laboratory experiment (Schmitt et al., 2008) with undisturbed soil columns, which were frozen three times for two weeks at different temperatures (- 3 °C, -8 °C and 13 °C) and irrigated with 80 mm water thereafter, demonstrated that intensive frost slightly enhanced the lignin mobilization in the O layer and its translocation into the B horizon. Furthermore, an increase of frost intensity strongly reduced the amount of hydrolysable plant and of microbial sugars in all soil horizons, probably through chemical alteration of sugar molecules leading to SOM stabilization. In addition, intensive freezing induced a switch of the structure of the microbial community, as the fungi are more susceptible to soil frost than bacteria, while the total sum of microbial biomass was not affected by increasing frost intensity.

2 Material and Methods

2.1 Experimental site

The experimental site (Coulissenhieb II) is a 140-years-old Norway spruce (*Picea abies* (Karst.) L.) located in the Fichtelgebirge mountains (Northern Bavaria, Germany) about 770 m above sea level. The annual temperature in the Fichtelgebirge area is +5.3 °C and the annual precipitation is 1,160 mm (Gerstberger et al., 2004). The soil has a sandy to loamy texture and is classified as Haplic Podzol according to the FAO soil classification derived from granite. The organic layer has a thickness of 6 – 10 cm, comprising of Oi, Oe, and Oa horizons and the mineral layers includes the horizons Ah, Bh, Bs, Bw and Bw/C (see Table 3-1 for basic soil properties). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

Table 3-1: Chemical properties of nine soil profiles in the Norway spruce stand at the Fichtelgebirge (n=9).

(CECeff = effective-cation-exchange capacity; BS = base saturation)

	Depth	pH		C	N	C : N	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Al^{3+}	H^{+}	CEC_{eff}	BS
	[cm]	(H_2O)	($CaCl_2$)	— [%] —			——— [$mmol_c\ kg^{-1}$] ———							[%]
O_a	5	4.0	3.3	18.0	1.0	17.7	94.4	7.6	1.4	3.4	74.1	8.0	197	54
Ah	-5	4.3	3.4	7.4	0.4	19.2	60.9	3.6	1.0	1.9	66.9	7.9	145	47
Bh	-12	4.3	3.4	5.5	0.3	19.7	70.9	2.3	1.1	1.3	97.6	4.0	185	41
Bs	-18	4.6	3.7	3.4	0.2	20.5	30.1	1.1	0.9	1.0	87.8	1.3	124	27
Bw	-55	4.6	4.1	1.3	0.1	12.3	3.7	0.2	1.1	1.0	41.3	0.4	48	12
Bw/C	<-55	4.5	4.0	0.4	0.0	8.3	2.7	0.2	3.0	1.1	35.3	0.2	43	16

2.2 Experimental design

Six plots, each of them measured 20 x 20 m, were established during the summer 2005, three as control and three as snow removal areas. All plots were prepared with instrumentation for measurement of soil temperature in the depths of –5, –15 and –25 cm beginning from the mineral soil surface and automatically recorded in 30 minutes intervals. In December 2005, the snow removal plots were covered with glass fibre nets of 0.5 cm mesh size protecting the forest floor from loss of litter layer due to the manual snow removal. After thawing in April 2006 the nets were removed. From late December 2005, before the first frost, the fresh-fallen snow was immediately removed by hand from the snow removal plots. After the end of the manipulation, the removed snow was not returned to the sites. For this reason, the snow removal plots received 147 mm less precipitation compared to the control plots. After the end of the snow removal period early February 2006, the snow was allowed to accumulate which isolated the soil frost until April 2006. The control plots were left undisturbed during the experiment and a snow cover of 50 – 80 cm accumulated at these plots from January 2006 to the end of March 2006.

2.3 Sampling

As the experimental field was designed for several years and different groups of researchers (gas measurements, DOC etc.), the plots should be disturbed by sampling so few as possible. As it could not be avoided that holes were produced with the sampling of the soil solid phase,

it became decided that with the installation of the measuring instruments in late spring 2005 the samples for the biomarker analysis (sugars and lignin) should be already taken for the winter experiment 2005/2006. Since one had assumed the control and SR plots did not differ significantly before the experiment and both plots experienced the same seasonal process. As reference for the PLFA analysis fresh samples were taken and kept frozen at –20 °C until analysis.

At the end of the freeze-thawing experiment in April 2006 (0406) 12 cores of soil (2 of each snow removal plot and 2 of each control for a mixed sample, 20 cm long and 7 cm diameter) were taken for the analysis of the biomarkers. In November 2006 (1106) 12 cores were taken again (2 from each plot for a mixed sample), to determine the long-term effects on SOM quality due to the preceding frost experiment.

All samples were divided into organic layer (0f + Oa horizon) and mineral soil (Ah + Bh horizon). The organic layer was cut into small pieces, mixed and the mineral horizon was also mixed and sieved < 2 mm. For PLFA analysis, an aliquot of fresh sample was kept frozen at –20 °C. For sugar and lignin analysis, the samples were dried at 40 °C and ground. For every horizon, the gravimetric water contents (dried at 105 °C) and the concentrations of total organic C (TOC) was analyzed using a EA-IRMS (MS: Finnigen Delta s, EA: Fisons EA1108).

2.4 Lignin analysis

The samples were oxidized with a modified alkaline CuO to release lignin-derived phenols (modified after Hedges and Ertel, 1982). Teflon-lined bombs were loaded with an equivalent of dry soil corresponding to 25 mg TOC, 25 µg ethyl vanillin (Fluka Chemie AG, 89555 Steinheim, Germany) in 1 mL 2 M NaOH (internal standard) and heated for 2 h at 170 °C on a platform shaker. After cooling to room temperature, the liquid was decanted into brown glass centrifuge tubes, the residue was washed with de-ionized water and centrifuged. The

supernatant was acidified to pH 1.8 – 2.2 and kept at room temperature in the dark (1 h) to precipitate humic acids being separated by centrifugation. An aliquot of 40 mL of the solution were put onto C_{18} – columns (Bakerbond speTM Octadecyl (C_{18}), J. T. Baker, reversed phase material, filling volume 3 mL, particle size 5 µm) and were eluted 9 times with 0.5 mL ethyl acetate, being concentrated subsequently by rotary evaporation, transferred with 25 µL phenyl acetic acid (recovery standard, Fluka Chemie AG, 89552 Steinheim, Germany) in 1 mL methanol to 2 mL glass reaction vials and dried under nitrogen gas.

For derivatization, the CuO oxidation products were re-dissolved in 100 µL pyridine p. a. (Merck, 64271 Darmstadt, Germany) and derivatized by reaction with 200 µL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature. For quantification, derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (further information Schmitt et al., 2008).

2.5 Sugar analysis

Plant- and microbial-derived sugar extraction was carried out according to a modified Amelung et al. (1996) method. Samples containing 8 mg TOC and 80 µg Myo-Inositol in de-ionized water as international standard and were hydrolyzed with 10 mL 4 M trifluoracetic acid flasks for 4 hours at 105 °C. After filtration through glass fiber filters (GF 6, Schleicher & Schüll, 37586 Dassel, Germany) the samples were dried using a rotary evaporator (60 hPa, water temperature 45°C). The sample was re-dissolved in 5 mL de-ionized water and put on top of a stacked Serdolit® PAD IV (5 g, Serva, 69115 Heidelberg, Germany) and 4 g dry Dowex® 50 W X 8 cation exchange resin. The columns were washed 5 times with 10 mL de-ionized water, collected and freeze-dried. The residue containing the saccharides were re-dissolved in de-ionized water and transferred into 3 mL glass reaction vials which were closed with parafilm and frozen until derivatization.

Derivatization was carried out according to a modified procedure described by Gross

and Glaser (2004). The frozen vials were freeze-dried and the sugars were re-dissolved in 100 µL dry pyridine p.a. (Merck KgaA, 64271 Darmstadt, Germany) containing 10 µg 3-O-Methylglucose (Sigma-Aldrich-Chemie, 82024 Taufkirchen, Germany) as recovery standard and in 450 µL dry pyridine p.a. containing 45 µg methyl boronic acid (MBA) (Sigma-Aldrich, 82024 Taufkirchen, Germany). The samples were heated at 60 °C for one hour and diluted with 450 µL ethyl acetate after cooling. The derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (further information Schmitt et al., 2008).

2.6 Phospholipid fatty acids (PLFA) analysis

PLFA extraction was carried out according to a modified Frostegard et al. (1991) method. To 5 g of thawed soil 18 mL extraction solution was added and shaken for 2 hours. After centrifugation (4000 rpm for 20 min) the supernatant was transferred into separating funnels. Subsequently, 15 µg PLFA 19:0 (internal standard, Biotrend, 50933 Cologne, Germany), 6.2 mL chloroform and 6.2 mL citrate buffer were added and shaken for 15 min. After separation over night, the organic extract (lower phase) was transferred into conical flasks, dried using a rotary evaporator, re-dissolved in chloroform and separated over glass columns filled with silica gel into neutral, glyco- and phospho- (polar) lipids (by elution with methanol). The methanol extract was dried using a rotary evaporator, transferred into 4 mL glass reaction vials with methanol and dried under a stream of nitrogen.

Fatty acid methyl esters (FAME) were prepared from free PLFA using a strong acid methylation. For this aim, the PLFA were re-dissolved in 0.5 mL 0.5 M NaOH in methanol, and heated at 100 °C for 10 minutes. After the addition of 0.75 mL boron trifluoride in methanol (concentration of BF3: 13 – 15%; Fluka, Seelze, Deutschland) the samples were heated at 80 °C for 15 minutes. After addition of 0.5 mL saturated NaCl solution, the samples were shaken 3 times 30 seconds with 1 mL hexane. The hexane phase was pipetted into

another reaction vial and dried under a stream of nitrogen. The PLFA were re-dissolved in 10 µL (5 µL for DOM) 13:0 FAME (Sigma-Adrich, 82024 Taufkirchen, Germany) in toluene as recovery standard and 490 µL (95 µL in case of DOM) toluene and transferred into GC auto sampler vials. The derivates were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID) (further information Schmitt et al., 2008).

Terminal-branched saturated PLFA (a15:0, i15:0, i16:0, i17:0, a17:0) are considered as makers for gram-positive bacteria while mid-chain branched saturated PLFA (10Me16:0, 10Me17:0, 10Me18:0) are associated with actinomycetes. Typical for gram-negative bacteria are monounsaturated fatty acids (16:1ω7c, 18:1ω7c) and cyclopropyl saturated PLFA (cy17:0, cy19:0). Short or odd-chain saturated PLFA (14:0, 15:0, 16:0, 17:0, and 18:0) are non-specific bacterial makers and are present in all microbial organisms. Typical markers for fungi are PLFA 18:2ω6,9, 18:1ω9c and 16:1ω5c (Zelles, 1999; Waldrop et al., 2004; McMahon et al., 2005).

2.7 Statistical analysis

Statistical analyses were carried out using STATISTICA 5.0. Differences between different treatments were evaluated using a one-way ANOVA followed by the Tukey-Honest post-hoc test.

3 Results and Discussion

3.1 Soil temperature

The mean air temperature from December 2005 until March 2006 were –3.8 °C being below a 10-years average of –1.5 °C mean winter temperature, whereas the following winter 2006/2007 was to mild (+1.2 °C mean winter temperature) (Goldberg et al., 2010). Before the beginning of our treatment in the middle of Decembers 2005, soil temperature (Figure 3-1)

was not different between the snow removal treatments and the control. Through the snow removal, the frost penetrated into the soil to about 15 cm depth, while the control remained unfrozen. At the snow removal treatments, the average soil temperature was –1.0 °C at 5 cm depth and –0.1 °C at 15 cm depth from 28th of December to 1st of April (Hentschel et al., 2009). At 25 cm soil depth, temperature was not different between the control and the frost-treated plots at any time (data not shown). In the middle of April 2006, the upper soil was completely thawed, while at 15 cm depth the soil temperature did not rise above 0 °C until end of April. Since May 2006 on, the soil temperature at the control was comparable to that of the snow removal treatments again.

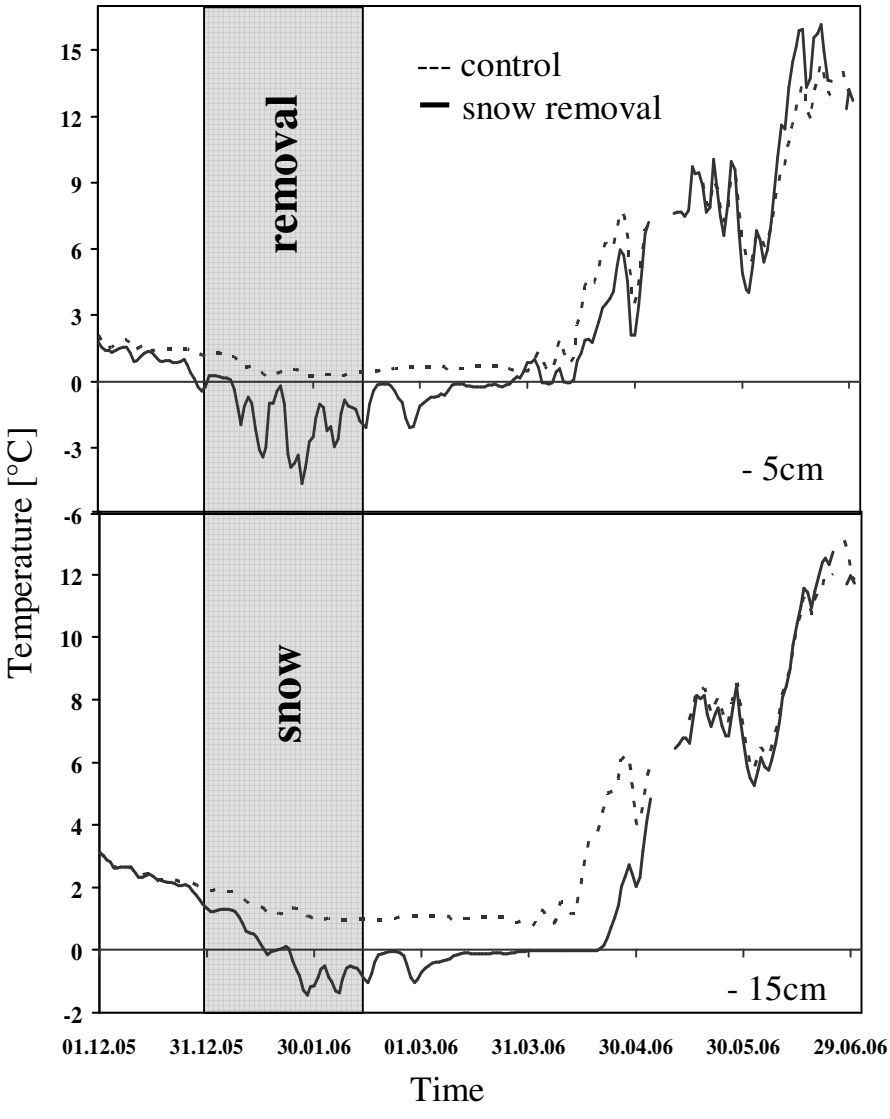

Figure 3-1: Soil temperature at the control and the snow removal plots in the soil depths of -5 cm and -15 cm.

3.2 Lignin phenols

The VSC lignin content ranged between 17 and 30 mg g^{-1} in the organic layer and between 6.1 and 12 mg g^{-1} in the mineral soil (Table 3-2), indicating advanced lignin decomposition with increasing soil depth which is in agreement with other studies on lignin distribution in forest soils (Koegel, 1986; Ziegler et al., 1986). The VSC lignin contribution to SOC ranged between 2 % and 5.3 % (Figure 3-2a) similar to other acid temperate forest soils (Koegel, 1986; Ziegler et al., 1986; Glaser et al., 2000; Spielvogel et al., 2007).

Table 3-2: Mean concentrations (± SE) of investigated biomarkers in the organic layer and mineral horizon before (2005) and after the snow removal (SR) experiment (0406), respectively after the following summer (1106) at the control and the SR plots.

	Control						**SR-treatments**					
	2005	SE	0406	SE	1106	SE	2005	SE	0406	SE	1106	SE
lignin [mg VSC g^{-1}]												
organic layer	24	± 1.2	37	± 4.4	53	± 1.7	31	± 2.0	33	± 3.2	51	± 2.3
mineral horizon	22	± 4.2	21	± 2.3	29	± 4.0	21	± 5.0	24	± 2.0	24	± 1.5
plant sugars [mg g^{-1}]												
organic layer	17	± 0.6	30	± 4.4	20	± 2.1	23	± 1.7	19	± 0.8	19	± 3.1
mineral horizon	18	± 5.5	14	± 2.3	8.1	± 1.9	11	± 2.6	14	± 2.8	6.2	± 0.7
microbial sugars[mg g^{-1}]												
organic layer	11	± 0.4	11	± 1.2	8.9	± 0.4	12	± 0.5	6.7	± 0.5	11	± 1.6
mineral horizon	12	± 2.5	9.7	± 1.4	7.2	± 2.1	8.5	± 1.3	8.4	± 1.8	6.1	± 1.2
sum of microbial biomass 21 PLFA [µg g^{-1}]												
organic layer	33	± 0.9	22	± 4.5	23	± 4.6	45	± 11	14	± 0.2	62	± 8.8
mineral horizon	n.a.		10	± 2.4	18	± 2.5	n.a.		12	± 2.2	51	± 10
21 PLFA [nmol kg^{-1}]												
organic layer	125	± 2.9	67	± 18	68	± 23	173	± 36	53	± 1.8	63	± 6.2
mineral horizon	n.a.		27	± 6.6	219	± 102	n.a.		39	± 1.8	188	± 66

In the organic layer of the control plots, the VSC lignin contribution to SOC (Figure 3-2a) significantly ($p < 0.05$) increased between May 2005 and April 2006 (over the period of the winter and snow removal experiment) and at both treatments between April 2006 and November 2006 (over the period of the following summer). This reflect the seasonal process with the litter entry and root growth and preferential mineralisation of easily accessible carbon compounds in spring and summer.

However, control and snow removal treatments did not differ in VSC lignin contribution to TOC in April 2006. Hence, the VSC lignin contribution to TOC (Figure 3-2a)

showed no clear frost effect in both horizons. Therefore, the results of the field experiment could not confirm our laboratory experiment (Schmitt et al., 2008), which demonstrated that intensive frost enhanced the lignin mobilization in the O layer and its translocation into the M horizon. A explanation for this phenomenon were the facts that (i) soil was still frozen at soil sampling and (ii) a throughfall of 147 mm was missing at the snow removal treatments. Thus, there was no way for increased lignin mobilization.

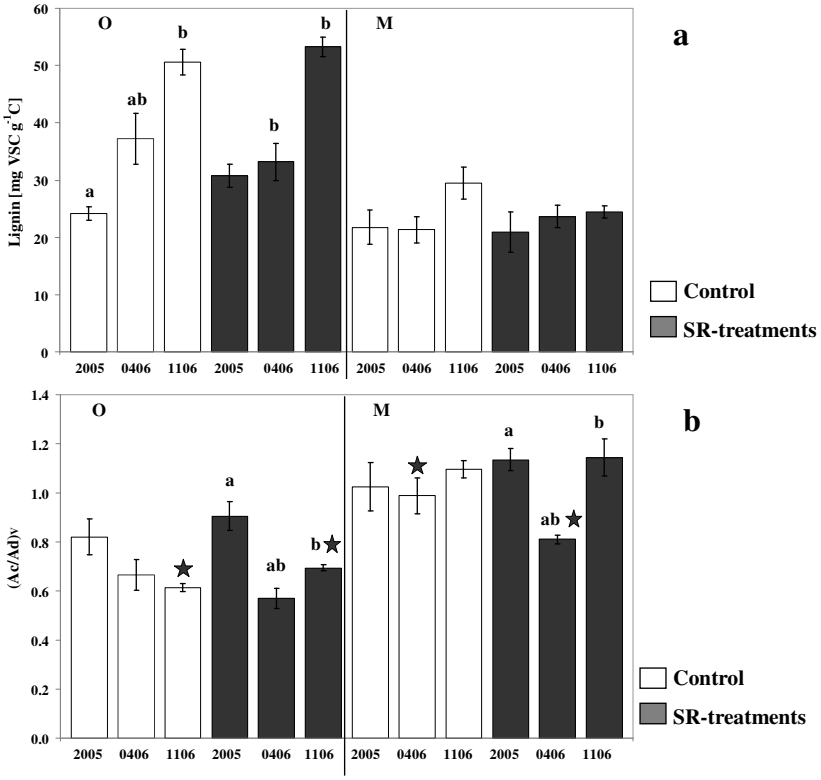

Figure 3-2: Mean value (± SE) of (a) lignin (sum of VSC) contribution to SOC [mg VSC g^{-1} C] and (b) ratio of acid to aldehyde of vanillin units [$(Ac/Ad)_v$] in the organic layer (O) and mineral horizon (M) before (2005) and after the snow removal (SR) experiment (0406), respectively after the following summer (1106) at the control (white bars) and the SR treatments (black bars). (✯ = significant ($p < 0.05$) differences between C and SR treatments, a = significant ($p < 0.05$) differences between sample day 2005 and 0406, b = significant ($p < 0.05$) differences between sample day 0406 and 1106).

The acid to aldehyde ratio of vanillyl monomers increased in the order O layer < mineral horizon (Figure 3-2b), due to progressive lignin decomposition in the same direction as typical for forest soils (Koegel-Knabner, 2000). Our results evidenced that vanillyl phenol degradation was retarded due to soil frost because the ratio of acid to aldehyde of the vanillyl monomers significantly decreased (< 0.05) at the snow removal treatments between Mai 2005

and April 2006 both in the organic layer and the mineral soil (Figure 3-2b). A slight decrease of the $(Ac/Ad)_V$ ratio with time could also be observed in the O horizon of the control site but differences were not significant. Explanations were (i) input of fresh litter dominated by aldehyde units or (ii) reduced mineralization during winter.

3.3 Plant and microbial sugars

The concentrations of the sum of plant sugars (arabinose + xylose) and microbial sugars (rhamnose and fucose) decreased in the order organic layer > mineral soil (Table 3-2), which is in line with literature data (Rumpel et al., 2002). The sum of total sugars in the soil ranged from 17 to 30 mg g^{-1} in the organic layer and 6 to 18 mg g^{-1} in the mineral soil. The sum of total sugars contribution to SOC (Data not shown) ranged between 1.2 to 4.2 %, the amounts are lower than in other forest soils (Glaser et al., 2000; Spielvogel et al., 2007). In the year 2005 before the beginning of the experiment, both plant and microbial sugar concentrations in the organic layer were lower on the control compared to the SR treatments while in the mineral soil the opposite was true (Figure 3-3). However, the ratio of plant to microbial sugars was not significantly different (Data not shown).

In the organic layer of the control, the sum of plant sugars contribution to SOC (Figure 3-3a) increased significantly ($p < 0.05$) between May 2005 and April 2006, whereas the sum of microbial sugars (Figure 3-3b) hardly changed. This reflect the seasonal trend with the litter entry and root growth in autumn and reduced decomposition during winter.

However, at the SR treatments both the sum of plant and microbial sugars contribution to SOC (Figure 3-3a and b) decreased significantly ($p < 0.05$) during the same time period. Decreasing concentrations of microbial sugars is an indication of reduced microbial activity under frost corresponding with the decreasing CO_2 respiration at the SR treatments. Muhr et al. (2009) reported the soil respiration fluxes from the snow removal treatments (around 1 mmol CO_2 m^{-2} h^{-1}) were almost constantly less than CO_2 fluxes of the control plots (1 – 3

mmol CO_2 m^{-2} h^{-1}) during the experiment.

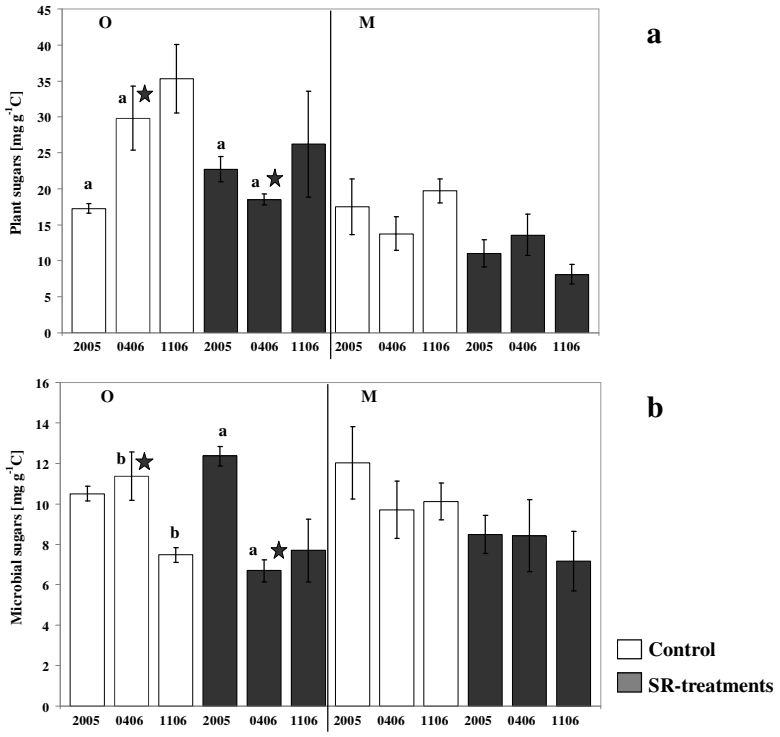

Figure 3-3: Mean value (± SE) of (a) plant and (b) microbial sugars contribution to TOC [mg g^{-1} C] in the organic layer (O) and mineral horizon (M) before (2005) and after the snow removal (SR) experiment (0406), respectively after the following summer (1106) at the control (white bars) and the SR treatments (black bars). (☆ = significant ($p < 0.05$) differences between C and SR treatments, a = significant ($p < 0.05$) differences between sample day 2005 and 0406, b = significant ($p < 0.05$) differences between sample day 0406 and 1106).

As microbial activity was reduced during the frost period, mineralisation could not be the reason for the significant ($p < 0.05$) disappearance of plant sugars. Elevated mobilization and translocation of the sugars with DOM as another process could also not be responsible, which was supported by the observation that DOM formed during winter and spring is dominated by carbohydrate-rich material from disrupted fresh microbial and plant debris (Kaiser et al., 2001), however an annual throughfall of 147 mm was missing at the snow removal treatments. In fact the continuing fine root production was nearly compensated through the fine root losses caused by low temperatures in the snow removal treatments (Gaul et al., 2008), however, this could also not be responsible for the significant ($p < 0.05$) differences between control and SR treatments.

In our laboratory experiment with undisturbed soil columns we determined that with

rising frost temperature the sum of plant sugar decreased (Schmitt et al., 2008). Sugar concentrations of an air-dried soil were higher than of the same soil (not air-dried) kept in a refrigerator (+4 to +8 °C, 4 weeks) or a freezer (-18 °C, 4 weeks). Therefore sugar production in less frozen soil can be ruled out as explanation for decreasing sugar concentrations with increasing frost intensity. Therefore we concluded, the only possible explanation for the disappearance of plant and microbial sugars upon soil freezing are chemical alteration of sugar molecules and/or stabilization (transformation of a labile into a stable SOM pool). These reasons are the cause here that the plant sugar decreased on the freezing areas contrary to TOC and sum of lignin. However, in our field experiment also reduced sugar exudation by rhizodeposition might be a reasonable explanation.

Between April 2006 and November 2006 at the SR- treatments the contents of plant sugars (Figure 3-3a) and microbial sugars (Figure 3-3b) in the organic horizon remained unchanged, thus freezing did not influence soil sugar dynamics in the longer term.

In the mineral horizon the frost was apparently not deep enough to induce reduced mineralization, physical changes of soil structure and/or organo-mineral stabilization of sugars. Muhr et al. (2009) measured still CO_2 respiration as the soil was frozen to a depth of 15 cm. Hence, in the mineral horizon, the content of plant and microbial sugars in contribution to TOC (Figure 3-3a and b) showed no effect of frost experience.

3.4 Phospholipid fatty acids (PLFA)

The sum of PLFA concentration ranged between 53 and 125 nmol kg^{-1} in the organic layer and between 27 and 219 nmol kg^{-1} in the mineral horizon similar to other acid temperate forest soils (Frostegard et al., 1993; Fierer et al., 2003b; Hackl et al., 2005), although different authors worked with a different number of PLFA. The sum of PLFA concentration in soil decreased in the order organic layer > mineral soil (Table 3-2). Before the beginning of the experiment, less microbial biomass was found at the control plots than at the snow removal

treatments (Table 3-2).

The contribution of PLFA to SOC (Figure 3-4a) increased in the order organic layer < mineral soil. PLFA contribution to TOC in our study was comparable to data from *Pinus sylvestris*, *Picea abies* and *Betula pendula* forest soils in Finland (Priha et al., 2001). Interestingly, the same authors also found higher microbial biomass contribution to TOC as measured by both chloroform fumigation extraction and PLFA concentrations in mineral soil horizons compared to organic layer. On the other hand, microbial activity was higher in the organic layer compared to the mineral soil as measured by respiration (Muhr et al., 2009) indicating that soil microbial biomass is dominant in mineral soil horizons but less active than in the organic layer.

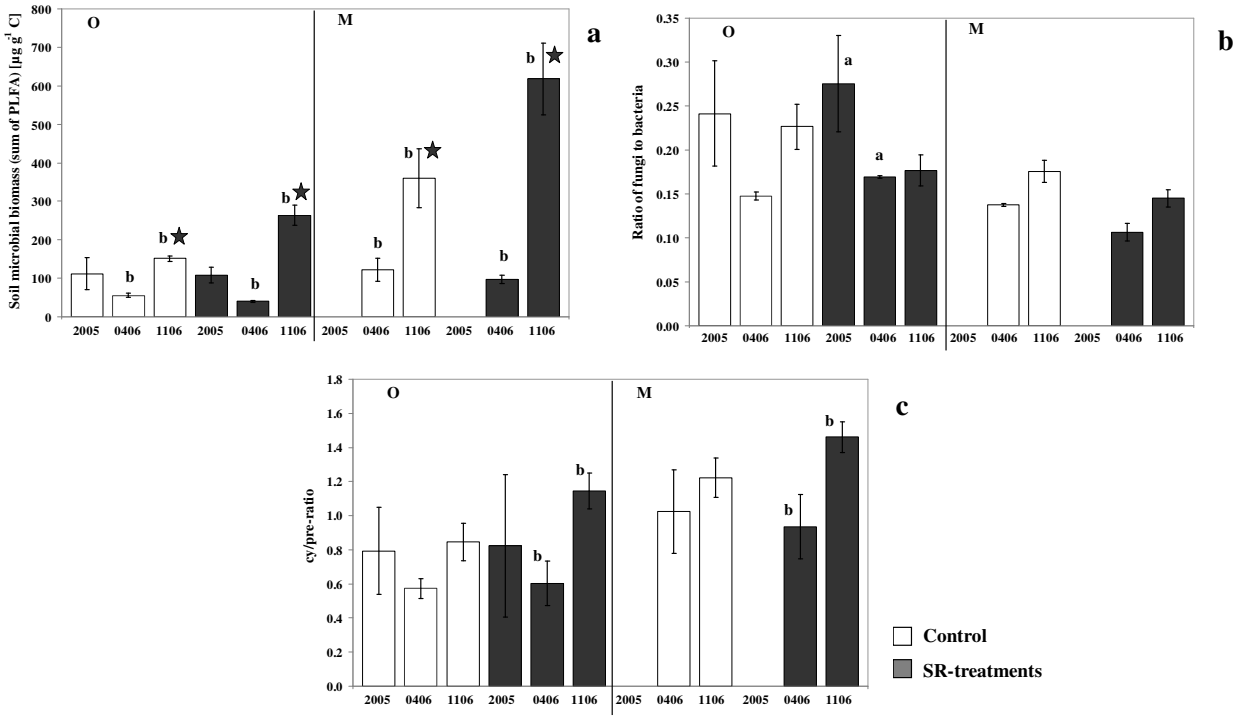

Figure 3-4: Mean value (± SE) of (a) microbial biomass (sum of PLFA) contribution to TOC [µg g^{-1} C], (b) ratio of fungal to bacterial PLFA and (c) the ratio of the sum of cyclopropyl PLFA to the sum of their monoenoic precursors [(cy17:0+cy 19:0)/(16:1ω7c+18:1ω7c; abbreviated as cy/pre)] in the organic layer (O) and mineral horizon (M) before (2005) and after the snow removal (SR) experiment (0406), respectively after the following summer (1106) at the control (white bars) and the SR treatments (black bars). (★ = significant ($p < 0.05$) differences between C and SR treatments, a = significant ($p < 0.05$) differences between sample day 2005 and 0406, b = significant ($p < 0.05$) differences between sample day 0406 and 1106).

In May 2005, the contribution of soil microbial biomass to TOC (Figure 3-4a) did not

differ between the control and the snow removal treatments and decreased between May 2005 and April 2006 over the period of the snow removal experiment both at the control and the snow removal treatments. Thus, no frost effect was observed with respect to soil microbial biomass. These results are consistent with former investigations showing that soil microbial biomass is obviously not affected by freeze/thaw events either in the laboratory (Schmitt et al., 2008) or under field conditions (Koponen et al., 2006). A explanation for this effect is the fact that microbes may adapt to freezing stress, at least in regions where soil frost occurs regularly (Neilson et al., 2001). Such tolerance has been also observed for example in alpine tundra or in Antarctic soils (Lipson et al., 2000). Also the tendency of decreasing cy/pre-ratio at the end of the snow removal experiment in April 2006 at both the control and snow removal treatments (Figure 3-4c) supported the assumption that soil microbial community did not suffer under moderate freezing stress.

Between April 2006 and November 2006 (over the period of the summer following the snow removal experiment) soil microbial biomass (sum of PLFA) increased significantly ($p < 0.05$) in the organic layer and mineral soil of both control and snow removal treatments (Figure 3-4a). This recovery of the micro organisms was a logical consequence of the temperature rise (Figure 3-1) in spring and summer 2006. However, in November 2006 due to higher input of TOC caused by frost and/or summer dryness like mortality of soil microbial biomass (Jenkinson and Powlson, 1976) or physical disruption of soil aggregates (Vangestel et al., 1992) the increase of the sum of PLFA was significant ($p < 0.05$) higher at the SR-treatments than at control (Figure 3-4a). In contrast, Muhr et al. (2009) observed 2006 an annual soil respiration flux of 5.1 t C ha^{-1} a^{-1} at the snow removal treatments and of 6.2 t C ha^{-1} a^{-1} at the control and the authors reported only 14% of the reduced soil respiration were attributed to the soil frost period itself, whereas 63% of this losses caused by the following summer. This results confirmed Feng et al. (2007), the authors ascertained the sum of microbial biomass was not closely related to soil microbial respiration but more strongly

controlled by substrate availability and quality.

At both treatments between May 2005 and April 2006 a decreased fungal to bacterial PLFA ratio was observed in the organic layer, but just at the SR treatments was this decreasing significant ($p < 0.05$). Thus, the frost treatment seems to affect fungi more than bacteria, a conclusion that is in accordance with literature findings (Feng et al., 2007; Schmitt et al., 2008). However a long-term effect to the structure of the microbial community could be observed, the fungi apparently suffered more under water stress induced through the frost experience, like bacteria, as the fungal to bacterial ratio did not increase again at the SR treatments like at control (Figure 3-4b). Also the significantly ($p < 0.05$) increasing cy/pre-ratio between April and November 2006 (Figure 3-4c) just at the SR treatments in both horizons confirmed a long-term environmental stress (i.e. suffered more from water stress) during the following summer for the soil microbial biomass.

4 Conclusions

TOC and lignin were not affected through the induced freezing event, while plant sugars decreased during the frost period which be explained by chemical alteration and stabilization with the mineral phase as already discussed for the preceding laboratory experiment. In the field experiment, also reduced rhizodeposition was a possible explanation. Also microbial sugars decreased during the frost experiment, due to reduced microbial activity. However, soil microbial biomass (PLFA) was not affected by the frost experiment indicating only effects on its soil respiration but not on their biomass corroborating rapid recovery upon quite natural phenomenon such as soil frost. Also a physiological stress indication of soil microbial biomass (cy/pre ratio) indicated a stress situation, however, this was observed not directly after the end of the frost experiment but in the longer term. In addition, soil fungi were more susceptible to soil frost as indicated by the ratio of fungal to bacterial PLFA.

Acknowledgements

This study was funded by the German Research Foundation within the Research Group "Soil processes under extreme climatic conditions" (BO 1741/3-2).

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Study 4

Organic matter dynamics in a temperate forest soil following enhanced drying

Andrea Schmitt [(a)] and Bruno Glaser [(a,b*)]

[(a)] Soil Physics Department, University of Bayreuth, D-95440 Bayreuth, Germany

[(b)] Current address: Soil Biogeochemistry, Martin-Luther-University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle, Germany.

[*] Corresponding author: bruno.glaser@landw.uni-halle.de, bruno.glaser@uni-bayreuth.de

Soil Biology and Biogeochemistry

Published 2011

Volumne 43

Pages 478 - 489

Abstract

Climate models predict an increase in global surface temperature and a change in precipitation intensity during this century. For Europe, extended drought periods followed by heavy rainfall are expected. The consequences for soil organic matter (SOM) dynamics are poorly understood. In this study, we investigated the effect of changing soil moisture regime on SOM quality under field conditions. For this purpose, a throughfall exclusion (TE) experiment was conducted in the summers 2006 and 2007 on a Haplic Podzol under a 140 years old Norway spruce stand using a roof installation followed by re-wetting compared to non-manipulated control plots. Total organic carbon, lignin (stable carbon pool), plant and microbial sugars (labile carbon pool) and microbial biomass (phospholipid fatty acids) were determined before, during and after the experiment in the L, O, A and B horizons. No significant treatment effects could be observed for SOM quantity. Amounts of lignin and soil microbial biomass were also not affected by the moisture regime but structure of soil microbial community. In the L and organic layers, gram+ bacteria and actinomycetes were reduced during water stress, while gram- bacteria, fungi and protozoa increased during drought. Warmer and drier weather led to a dominance of fungi while a cooler and moister regime favoured bacteria, at least in the L horizon. An increasing PLFA (cy17:0+cy19:0)/ (16:1ω7c+18:1ω7c) ratio in the O layer and A horizon suggests that the microbes suffered from water stress in these horizons. This agrees with a decreasing contribution of microbial sugars to SOM with decreasing water content in the O and A horizons. Although the original plant material exhibited increasing plant sugar content with increasing dryness, the contribution of the plant sugars to total soil organic carbon (SOC) generally decreased with decreasing water content. Physical-chemical changes of soil structure can theoretically change the sugar extractability from soils and/or chemical changes of sugars structure can probably affect the analysis. Therefore,

chemical alteration and stabilization could be responsible for sugar decrease in soil with increasing dryness explaining the contrast compared to the original plant material.

Keywords: climate change, disturbance, soil organic matter, biomarker, lignin, sugar, microbial community, phospholipid fatty acid, drought

1 Introduction

Climate models predict an increase in global surface temperature and a change in precipitation intensity during this century. In the basic scenario for the period 2004 – 2055, the global mean air temperature will increase between 0.6 and 1.5 °C (Gerstengarbe and Werner, 2008). For Northern Bavaria, the IPPC climate change scenario A1B prognoses an increase of the mean annual temperature between 3 °C and 3.5 °C for the period of 2071 – 2100 compared to the reference period 1961 – 1990 (Beierkuhnlein and Foken, 2008). As a consequence, mean annual precipitation will increase where precipitation is high today, and in regions with low precipitation it will further decrease. Furthermore, the sum of precipitation will increase in winter and decrease in summer (Gerstengarbe and Werner, 2008). Additionally, in Germany the number of summer days with a maximum temperature ≥ 25 °C and days with heavy precipitation (≥ 10 mm) will increase.

A shift in amount, intensity and frequency of precipitation could change the storage of C in temperate forest ecosystems (Borken and Matzner, 2009). Under the impact of drying and re-wetting, soils undergo complex changes of soil structure (aggregation), soil organic matter (SOM) and microflora compositions (Denef et al., 2001). Soil drying and re-wetting could also cause a significant stress on soil microbial community and induce significant changes in microbial C dynamics (Fierer and Schimel, 2002).

Dryness of soil is often linked to occurrence of soil water repellency (Doerr and

Thomas, 2000) which diminishes with time when the material is wetted (Lamparter et al., 2009). In soils or organic material with good wettability, the hydrophilic functional groups of OC are exposed to the outside, whereas in water-repellent material hydrophobic functional groups can be found at the outside (Diehl et al., 2009). Hydrophobic properties of SOM have multiple effects on stabilization mechanisms, namely protection of labile molecules, enhancement of aggregate stability. Furthermore, hydrophobicity reduces surface wettability and thus the accessibility of OM for microorganisms (von Lutzow et al., 2006). This is supposed to result in decreased decomposition rates. Soil microbial biomass and thus microbial activity can increase again with rainfall (Cosentino et al., 2006).

Numerous studies exist on the effects of drying and re-wetting on C cycle. The C turnover affected by drying was well examined, but attention was mainly directed towards drying-re-wetting effects on dissolved organic carbon (DOC), respiration (CO_2) or total soil organic carbon (TOC, SOC). DOC production accelerate under warmer and drier conditions (Christ and David, 1996; Tipping et al., 1999), but in mineral soils, leaching depends also on adsorption. While Lammersdorf et al. (1998) observed a DOC peak after re-wetting, (Borken et al., 1999; Hentschel et al., 2007) could not confirm this observation. Consensus exists on decreased soil CO_2 respiration during drying while a CO_2 pulse was reported after re-wetting (Borken et al., 2003; Fierer and Schimel, 2003; Wu and Brookes, 2005). However, other authors could not observe this CO_2 pulse, especially under field conditions (Degens and Sparling, 1995; Muhr et al., 2008). The dynamics of C mineralisation in soils depends both on chemical properties, like pH and C/N ratio and on physical properties, like aggregate distribution and moisture regime (Lamparter et al., 2009).

However, SOM is comprised of ecologically and chemically different pools such as labile (active), intermediate (slow) and stable (passive) pools. The active or labile SOM pool is dominated by sugars, organic acids and soil microbial biomass with a mean residence time (MRT) of days to months (Cochran et al., 2007). The intermediate or slow SOM pool

comprises structural plant residues with a MRT of 25 – 50 years. Lignin phenols are more stable against microbial decomposition and contribute mainly to the long-term C pool of forest floors (Haider, 1996), with a mean residence time of 1,000 – 1,500 years (Cochran et al., 2007). However, this doctrine is also disputed ((Kiem and Kogel-Knabner, 2003; Dignac et al., 2005). (Heim and Schmidt, 2007) detected a lignin turnover of 5 – 26 years in grassland and 9 – 38 years in arable soils, thus being in the same order of magnitude as sugars. The two-reservoir-model of Rasse et al. (2006) indicated that chemical recalcitrance alone is not sufficient to explain lignin turnover in soils and the most relevant mechanisms appear to be transfer of lignin molecules and fragments from decomposing plant tissue to protected soil fractions. However, all of these authors worked with soils of European agro ecosystems without organic layer and cultivated plants like maize. Hence, it is difficult to transfer these results to a forest soil with a spruce stock. For these reasons, we still consider lignin as a stabile C pool in forest ecosystems due to specific structural properties, as mentioned in Haider (1996) and Cochran (2007).

Biomarker profiles provide chemical information about changes in soil microbial community structure or in substrate use after environmental disturbance (Sulkava and Huhta, 2003; Borjesson et al., 2004). The close relationship between soil microbial community composition and soil processes suggests that changes in the ecosystem through environmental stress such drying/re-wetting events shift microbial community composition (Frostegard et al., 1993; Balser and Firestone, 2005). The ratio of fungal/bacterial phospholipids fatty acids (PLFA; fungi/sum of gram-positive bacteria + actinomycetes + gram-negative bacteria) can be used as indicator for nutritional stress (Fierer and Schimel, 2003).

A laboratory experiment (Schmitt et al., 2010) with undisturbed soil columns, which were dried three times for five weeks and re-wetted with different raining intensity (8 mm, 20 mm and 50 mm), demonstrated that (a) lignin was not systematically affected in the O horizon through drying and re-wetting; (b) an increase of dry / wet intensity strongly reduced the

amount of hydrolysable plant and microbial sugars in all soil horizons, probably through chemical alteration of hydrolysable sugar molecules in organic and mineral soil horizons and (c) drying and intensive re-wetting induced a switch of soil microbial community structure in mineral soil and soil solution but the amount of soil microbial biomass was not altered.

Nevertheless, there are hardly investigations regarding the changes of SOM composition upon drying events under field conditions. Hence, the focus of this work was to characterize different SOM pools in a drying / re-wetting field experiment by means of the biomarkers lignin (stable pool), plant- and microbial derived sugars (labile pool) and PLFA as measure for the microbial biomass and its community structure.

2 Material and Methods

2.1 Experimental site

Table 4-1: Chemical properties of the Haplic Podzol under Norway spruce at the Fichtelgebirge throughfall exclusion experiment.

	Thickness	pH		BD	RF	C	C stock	C/N	CEC_{eff}	BS
	[cm]	H_2O	$CaCl_2$	[g cm^{-3}]	[V-%]	[%]	^{2}]		kg^{-1}]	%
O_a	4.9	4.0	3.3	0.3	7	21.2	2.0	19	197	54
EA	5.2	4.3	3.4	0.6	11	8.3	2.6	21	145	47
Bsh	5.3	4.3	3.6	0.8	15	6.0	2.4	22	185	41
Bs	11.4	4.6	3.8	0.8	18	3.6	2.7	21	124	27
Bv	30.5	4.6	4.2	1.2	25	1.4	4.4	8	48	12

BD = bulk density of fine earth
RF = volumetric rock fraction

The experimental site (Coulissenhieb II) is a 140-years-old Norway spruce (*Picea abies (Karst.) L.*) stand located in the Fichtelgebirge mountains (Northern Bavaria, Germany) at about 770 m above sea level. The mean annual temperature in this area is +5.3 °C and the mean annual precipitation is 1,160 mm (Gerstberger et al., 2004). The soil has a sandy to loamy texture and is classified as Haplic Podzol according to the FAO soil classification and the parent material of the soil is granite. The organic layer consists of Oi, Oe and Oa horizons

and has a thickness of 8 to 12 cm. The mineral layer includes the horizons EA, Bsh, Bs, Bw (see Table 4-1 for basic soil properties). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

2.2 Experimental design

Three control (C) plots and three throughfall exclusion (TE) plots were established. During summer 2005, wooden roof structures of about 400 m² (20 x 20 m) and 3 m height were constructed at the TE sites. Between 22nd June and 8th August 2006, the roof construction was covered with transparent plastic panels (Owolux 76/18, 1140 x 2500 mm) to simulate summer dryness as the precipitation water was kept out. Litter on the roofs was collected during the TE period and applied after the end of the experiment to the soil surface again. Throughfall exclusion rainwater was drained off using pipes to a distance of at least 35 m from the experimental area. Through the roof construction, about 70 mm throughfall was excluded during the TE experiment. After the end of the experiment, the same amount of artificial throughfall solution was applied via a sprinkler system within 2 days. After re-wetting, the plastic panels were removed. Between 3rd July and 13th August 2007 the drying experiment was repeated. However, this time the TE plots were not re-wetted at the end of the manipulation.

On 18th January 2007, the heavy low pressure system Kyrill moved over our experimental site with almost no destruction at the TE plots, while at the control plots, some trees were pulled out including the roots, thus destroying the soil structure. Although trees were also removed subsequently at the TE plots for homogenization purposes, the interpretation of the results of the year 2007 and the comparability with the control sites have thus to be treated with caution.

2.3 Sampling

As the experimental field was designed for several years and different groups of researchers (gas measurements, DOC etc.), the plots should be disturbed by sampling as few as possible. As it could not be avoided that holes were produced with the sampling of the soil solid phase, it was decided that control samples for the 2006 experiment were already taken a few weeks after the installation of the measuring instruments in 2005. At the end of the drying/re-wetting experiment in August 2006, 12 soil cores (2 of each plot for a mixed sample, 20 cm long and 7 cm diameter) were taken from the TE plots and from the untreated controls for biomarker analysis again. In 2007, before the beginning (June 2007) and at the end (August 2007) of the second drying experiment, cores were taken again from each plot.

All samples were separated into organic layer (L and O horizons) and mineral soil (A and B horizons). The organic horizons were cut into small pieces and mixed and the mineral horizons were sieved < 2 mm. The samples were dried at 40° C and ground for sugar and lignin analyses. For PLFA analysis, an aliquot of fresh samples was kept frozen < –20 °C until analysis. For every horizon, the gravimetric water contents (dried at 105 °C) and the concentration of total organic C (TOC) were analyzed using elemental analysis (Fisons EA1108).

2.4 Lignin analysis

Lignin is a main component of forest litter and represents a major input of organic matter into forest soils (Ziegler et al., 1986). Lignin compounds are phenolic polymers consisting of vanillin (V), syringyl (S), and cinamyl (C) moieties occuring in the cell walls of all vascular plants (Hedges and Ertel, 1982). The sum of V+S+C (VSC) after alkaline CuO oxidation is an indicator of the amount of intact lignin moieties. However, a quantification of lignin in soil is not possible with this and other methods (Ziegler et al., 1986), which might be due to the complex structure of lignin in combination with other organic compounds such as cellulose.

Nevertheless, the alkaline CuO oxidation method releasing phenols from reactive sites of the lignin macromolecule is a relative measure for the lignin content in soils (Amelung et al., 1999).

The samples were oxidized with alkaline CuO to release lignin-derived phenols (modified after Hedges and Ertel, 1982). Teflon-lined bombs were loaded with an equivalent of dry soil corresponding to 25 mg TOC, 25 µg ethyl vanillin (Fluka Chemie AG, 89555 Steinheim, Germany) in 1 mL 2 M NaOH (internal standard) and heated for 2 h at 170 °C on a platform shaker. After cooling to room temperature, the liquid was decanted into brown glass centrifuge tubes, the residue was washed with de-ionized water and centrifuged. The supernatant was acidified to pH 1.8 – 2.2 and kept at room temperature in the dark (1 h) to precipitate humic acids being separated by centrifugation. An aliquot of 40 mL of the solution was put onto C_{18} – columns (Bakerbond spe^{TM} Octadecyl (C_{18}), J. T. Baker, reversed phase material, filling volume 3 mL, particle size 5 µm), which were eluted 9 times with 0.5 mL ethyl acetate, being concentrated subsequently by rotary evaporation, transferred with 25 µL phenyl acetic acid (recovery standard, Fluka Chemie AG, 89552 Steinheim, Germany) in 1 mL methanol to 2 mL glass reaction vials and dried under nitrogen gas.

For derivatization, the CuO oxidation products were re-dissolved in 100 µL pyridine p. a. (Merck, 64271 Darmstadt, Germany) and derivatized by adding 200 µL N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) at room temperature. For quantification, derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). For fu(Further information Schmitt et al., 2008). The recovery of an internal standard ranged between 53 – 88%.

2.5 Sugar analysis

Non-cellulosic sugars are important SOM constituents being most abundant in root exudates and non-structural plant compartments (Derrien et al., 2004). While the pentoses arabinose

and xylose are mainly plant-derived, hexoses and deoxy sugars such as fucose and rhamnose are of microbial origin (Gross and Glaser, 2004). Plant DNA contains deoxy ribose but not rhamnose and fucose, the latter two being analysed in our study as microbial markers. Advanced biodegradation of SOM shifts the sugar composition from pentoses to deoxy sugars indicating consumption of plant-derived organic matter and production of microbial compounds (Murayama, 1984; Kaiser et al., 2004). The ratio of arabinose + xylose to fucose + rhamnose is a tool to trace effects of environmental changes on SOM degradation (Oades, 1989; Guggenberger and Zech, 1994; Amelung et al., 1999; Glaser et al., 2000).

Plant- (arabinose + xylose) and microbial- (fucose + Rhamnose) derived sugar extraction from soil and freeze-dried material was carried out according to a modified Amelung et al. (1996) method. Samples containing 8 mg TOC and 80 µg Myo-Inositol in de-ionized water as internal standard were hydrolyzed with 10 mL 4 M trifluoracetic acid for 4 hours at 105 °C. After filtration through glass fiber filters (GF 6, Schleicher & Schüll, 37586 Dassel, Germany) the samples were dried using a rotary evaporator (60 hPa, water temperature 45 °C). The samples were re-dissolved in 5 mL de-ionized water and put on top of a stacked Serdolit® PAD IV (5 g, Serva, 69115 Heidelberg, Germany) and 4 g dry Dowex® 50 W X 8 cation exchange resin. The columns were washed 5 times with 10 mL de-ionized water, collected and freeze-dried. The residue containing the saccharides were re-dissolved in de-ionized water and transferred into 3 mL glass reaction vials which were closed with parafilm and frozen until derivatization.

Derivatization was carried out according to a modified procedure described by Gross and Glaser (2004). The frozen vials were freeze-dried and the sugars were re-dissolved in 100 µL dry pyridine p.a. (Merck KgaA, 64271 Darmstadt, Germany) containing 10 µg 3-O-Methylglucose (Sigma-Aldrich-Chemie, 82024 Taufkirchen, Germany) as recovery standard and in 450 µL dry pyridine p.a. containing 45 µg methyl boric acid (Sigma-Aldrich, 82024 Taufkirchen, Germany). The samples were heated at 60 °C for one hour and diluted with 450

µL (100 µL for freeze-dried DOM) ethyl acetate after cooling. The derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). For (Further information Schmitt et al., 2008).

2.6 Phospholipid fatty acids (PLFA) analysis

Phospholipid fatty acids (PLFA) are membrane components of all living micro organisms but do not occur in microbial storage products (Zelles et al., 1992; Zelles et al., 1994; Cifuentes and Salata, 2001). Therefore, PLFA content is a measure for soil microbial biomass correlating with methods commonly used such as substrate-introduced respiration (Zelles et al., 1994; Zelles, 1999; Baath and Anderson, 2003), total aldehyde content (e.g. Zelles, 1999), chloroform-fumigation extraction in mineral soils (e.g. Bailey et al., 2002) and in forest floors (Leckie et al., 2004). After death of the microorganisms, PLFA are rapidly decomposed.

Individual PLFA are characteristic for different microbial groups (Frostegard et al., 1993; Cavigelli et al., 1995; Zelles, 1999). Terminal-branched satured PLFA (a15:0, i15:0, i16:0, i17:0, a17:0) were considered as makers for gram-positive bacteria and mid-chain branched saturated PLFA (10Me16:0, 10Me17:0, 10Me18:0) were associated with actinomycetes. Typical for gram-negative bacteria are monounsaturated fatty acids (16:1ω7c, 18:1ω7c) and cyclopropyl saturated PLFA (cy17:0, cy19:0). Short or odd-chain saturated PLFA (14:0, 15:0, 16:0, 17:0, and 18:0) were considered as non-specific bacterial makers and are present in all microbial organisms. Typical markers for fungi are PLFA 18:2ω6,9, 18:1ω9c and 16:1ω5c (Stahl and Klug, 1996; Zelles, 1999; Myers et al., 2001; Ruess et al., 2002; DeForest et al., 2004; Waldrop et al., 2004; McMahon et al., 2005).

Phospholipid fatty acid extraction was carried out according to a modified Frostegard et al. (1991) method. To 5 g fresh soil, 18 mL extraction solution was added [1 : 2 : 0.8

chloroform : methanol : citrate buffer solution (6.3 g citric acid monohydrate in 200 mL de-ionized water and with potassium hydroxide pellets adjusted to pH 4.0)] and shaken for 2 hours. After centrifugation, the supernatant was transferred into separating funnels. Subsequently, 15 µg PLFA 19:0 (internal standard, Biotrend, 50933 Cologne, Germany), 6.2 mL chloroform and 6.2 mL citrate buffer were added and shaken for 15 min. After separation over night, the organic extract (lower phase) was transferred into conical flasks, dried using a rotary evaporator, re-dissolved in chloroform and separated over glass columns filled with silica gel into neutral, glyco- and phospho- (polar) lipids. The methanol extract containing PLFA was dried using a rotary evaporator, transferred into 4 mL glass reaction vials with methanol and dried under a stream of nitrogen.

Fatty acid methyl esters (FAME) were prepared from free PLFA using a strong acid methylation. For this aim, the PLFA were re-dissolved in 0.5 mL 0.5 M NaOH in methanol, and heated at 100 °C for 10 minutes. After the addition of 0.75 mL boron trifluoride in methanol (concentration of BF_3: 13 – 15%; Fluka, Seelze, Deutschland), the samples were heated at 80 °C for 15 minutes. After addition of 0.5 mL saturated NaCl solution, the samples were shaken 3 times for 30 seconds with 1 mL hexane. The hexane phase was pipetted into another reaction vial and dried under a stream of nitrogen. The PLFA were re-dissolved in 10 µL 13:0 FAME (Sigma-Adrich, 82024 Taufkirchen, Germany) in toluene as recovery standard and 490 µL toluene and transferred into GC auto sampler vials. The derivatives were analyzed by capillary gas chromatography equipped with a flame ionization detector (GC-FID). For f(Further information Schmitt et al., 2008).

2.7 Statistical analysis

Statistical analyses were carried out using STATISTICA 5.0. Differences between different treatments were evaluated using a one-way ANOVA followed by the Tuckey-Honest post-hoc test. Correlation coefficients were computed with Excel 2003.

3 Results and Discussion

3.1 Soil moisture

During the experiment, the matric potential beneath the Oa horizon varied between -0.01 MPa (pF 2, moist) and -100 MPa (pF 6, very dry; Figure 4-1a). In 2006, in the organic layer of the TE plots the matric potential decreased from -0.1 MPa (pF 3) to -100 MPa (pF 6). However, due to a coinciding natural drought during our experiment, the control plots were also very dry (-10 MPa, pF 5). During summer 2007, no water stress was observed at the control plots (ca. 0.1 MPa, pF 3), whereas the TE plots experienced severe drought again (ca. -1 MPa, pF 4).

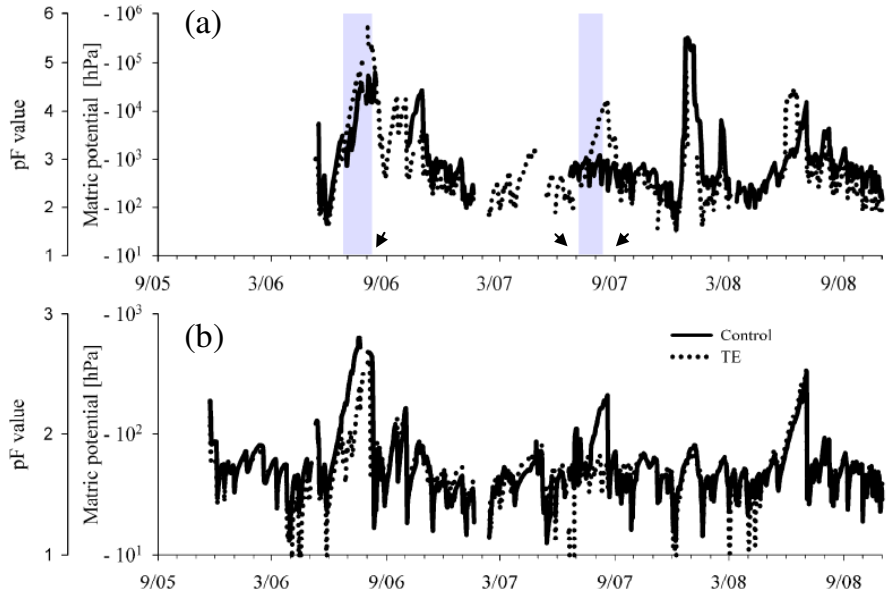

Figure 4-1: Matric potential beneath (a) the Oa horizon and (b) in 20 cm soil depth from September 2005 to November 2008 (Sampling day is indicated by ↓).

The matric potential in 20 cm soil depth was only slightly affected by drought in both manipulation years (Figure 4-1b). During the induced soil drought from late June to early August 2006, the soil matric potential in 20 cm depth decreased from -200 hPa (pF 2.3) to minimum values of -650 hPa (pF 2.8) at the TE plots and reached initial values within two days because of re-wetting. At the control plots, a similar trend could be observed, as the TE experiment was conducted at a natural drought in June/July. However, the matric potential of

about -400 hPa (pF 2.6) was significantly ($p < 0.05$) lower in the TE plots compared to the control plots. In the wet summer of 2007, matric potential on the control plots was around -50 hPa (pF 1.7) compared to a minimum soil matric potential of -200 hPa (pF 2.3) on the TE plots.

The gravimetric water content generally decreased in the order O layer > A horizon > B horizon (Figure 4-2). The L horizon showed no clear trend. After re-wetting in August 2006, the gravimetric water content at the TE plots was equal to the level of the controls again, with exception of the organic layer. An explanation for this could be the fact that the flow regime switched to predominantly inhomogeneous preferential flow in macro pores such as root channels so that the surrounding soil is not necessarily moistened (Hentschel et al., 2009). Hence organic matter mobilization is also a local process, it will depend on local flow conditions associated with different surface and subsurface pathways, on spatial heterogeneities in soil properties, soil moisture conditions, vegetation cover, rainfall intensities and surface and subsurface flow rates (Beven et al., 2005).

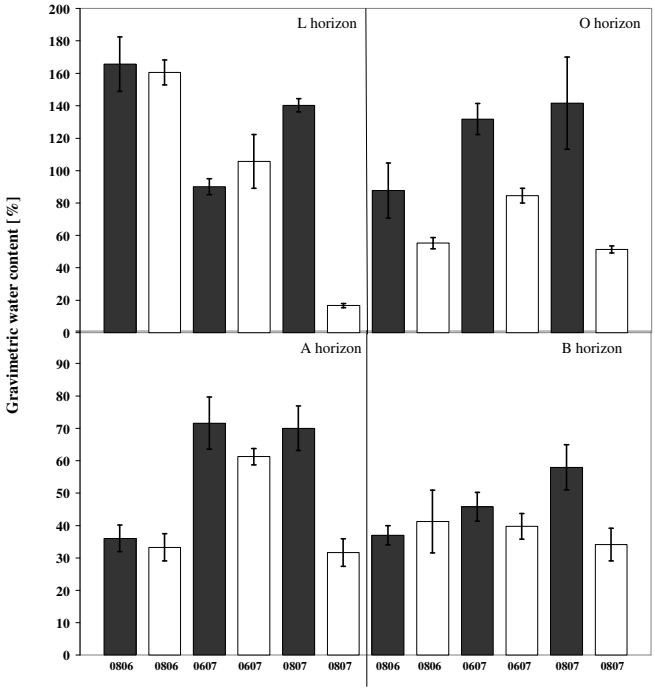

Figure 4-2: Mean gravimetric water content (%± SE) before (05/2005) and after (08/2006) the drying-re-wetting experiment 2006, respectively before (06/2007) and after (08/2007) the drying experiment 2007 at control (black bars) and TE plots (white bars).

Between June and August 2007, the gravimetric water content significantly decreased ($p < 0.05$) in the L, O and A horizons of the TE plots (Figure 4-2). During the same period, the water content at the control plots remained more or less unchanged or increased, in the L horizon even significantly ($p < 0.05$) indicating that our drying experiment was successful. However, as in 2007 -0.1 MPa (pF 3) was never exceeded (Fig 1B), this experiment could be classified only as a moderate drought.

3.2 Kyrill

In January 2007, the storm Kyrill caused large damages at our control plots whereas the TE sites were less influenced. Chapman et al. (2008) postulated that disturbance from hurricane Katrina influenced structure and composition of coastal forests through species-specific differences in damage and mortality rates. Such natural ecosystem disturbances induce also effects of soil compaction, mechanical destruction of forest floor, tree harvesting and the mechanical transfer of forest floor material into mineral topsoil (Spielvogel et al., 2007). Whole root bundles were pulled out and thus the soil structure was partly destroyed, also within ranges, which work at first sight unaffected, jerky tearing to the roots might have consequences for example for soil packing. We decided also to cut down trees on TE plots for better comparability. In the neighbouring experimental area (Coulissenhieb I) Kalbitz et al. (2004) conducted a clear-cutting experiment. The authors found an increasing decomposition and transformation of more decomposed organic matter with increasing contribution of lignin-derived compounds to the released organic matter probably due to increased microbial activity due to increased temperature of about 1.5 °C after clear-cutting. Also leaching of DOM increased with increasing water fluxes. In our case, only some trees were removed for better comparability with more Kyrill-disturbed control sites. Muhr et al. (2009), Goldberg and Gebauer (2009) and Schulze et al. (unpublished) also repeated the experiment at Coulissenhieb II in 2007, stating that the cause of the changing gas fluxes and DOC export

was primarily due to the drought experiment.

However, a comparison of the control 2007 with the control 2006 or with the TE plots was critical, reflected by our data. For example, the amounts of total sugars [mg g^{-1}C] did not correlate with the water content when all data are considered (Figure 4-3a). However, removing the control plots 2007 (after Kyrill) yielded significant correlation coefficients (Figure 4-3b). These data clearly indicate that Kyrill influenced our control plots more than the TE plots and that our homogenization effort did not fully compensate for the Kyrill damage. Therefore, for the following analysis, only control plots data before Kyrill were used (control 0806).

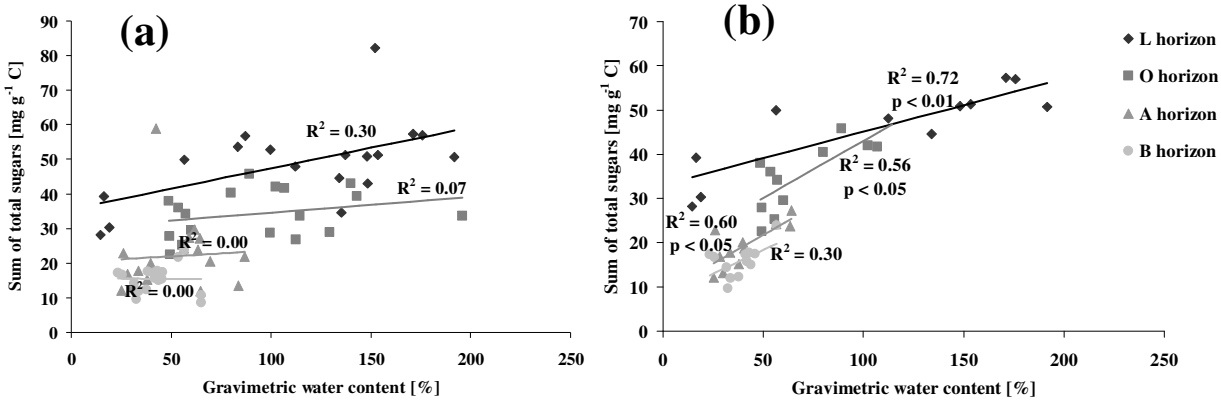

Figure 4-3: Correlation between gravimetric water content [%] and the sum of sugars related to SOC [mg g^{-1} C] (a) all plots 2006 and 2007 and (b) without control 06/2007 and 08/2007.

3.3 Lignin phenols

Lignin concentrations referred to TOC generally decreased with increasing soil depth (L horizon > O layer > A horizon > B horizon (Figure 4-4a), indicating advanced lignin decomposition in the same direction being in agreement with other studies on lignin distribution in forest soils (Koegel, 1986; Ziegler et al., 1986). The VSC lignin contribution to SOC ranged between 1.3 % and 5.7 % similar to other acid temperate forest soils (Koegel, 1986; Ziegler et al., 1986; Glaser et al., 2000; Spielvogel et al., 2007). The acid-to-aldehyde ratio of vanillyl monomers (Figure 4-4b) increased in the order L horizon < O layer < A horizon < B horizon, due to progressive decomposition being typical for forest soils (Koegel-

Knabner, 2000).

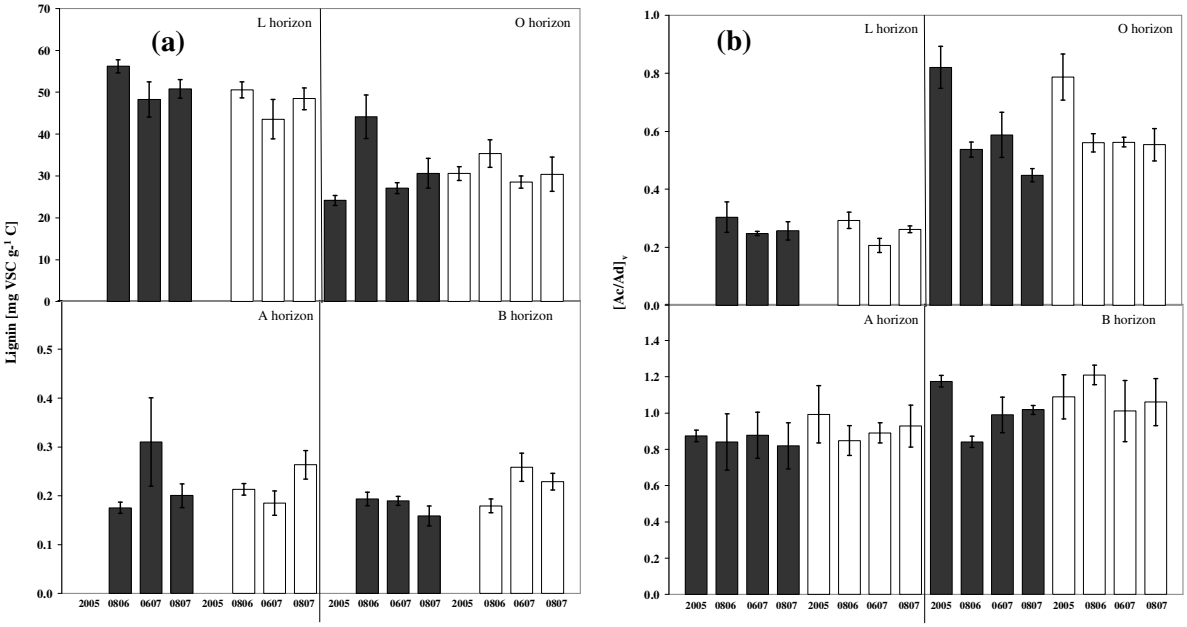

Figure 4-4: Mean value (± SE) of (a) lignin [mg VSC g^{-1} SOC] and (b) ratio of acid to aldehyde of vanillyl unit before (05/2005) and after (08/2006) the drying-re-wetting experiment 2006, respectively before (06/2007) and after (08/2007) the drying experiment 2007 at control (black bars) and TE plots (white bars).

Changes of VSC lignin contribution to SOC did not differ between the control and the TE plots (Figure 4-4a) and therefore, lignin was obviously not affected by drought and re-wetting. This result was also confirmed by the lacking correlation between VSC lignin contribution to SOC and the gravimetric water content (Figure 4-5a).

Gymnosperm lignin consists of 80 % of coniferyl alcohol-derived units (vanillyl phenols) (Ziegler et al., 1986) and the lignin molecule is typically altered during decomposition by white-rot fungi oxidizing aldehyde units producing acid units (Koegel, 1986). Therefore, the ratio of the oxidized derivatives versus the corresponding aldehyde $[Ac/Ad]_v$ of the vanillyl monomers is an indicator for the degree of degradation (Koegel-Knabner, 2000; Otto and Simpson, 2006). However, Figure 4-4b and the lacking correlation between $[Ac/Ad]_v$ and the gravimetric water content (Figure 4-5b) evidenced that the degradation of vanillyl phenols were not affected through the drought experiment. Thus, lignin can be considered as a relatively stable SOM pool in forest soils, even under moderate

climate change.

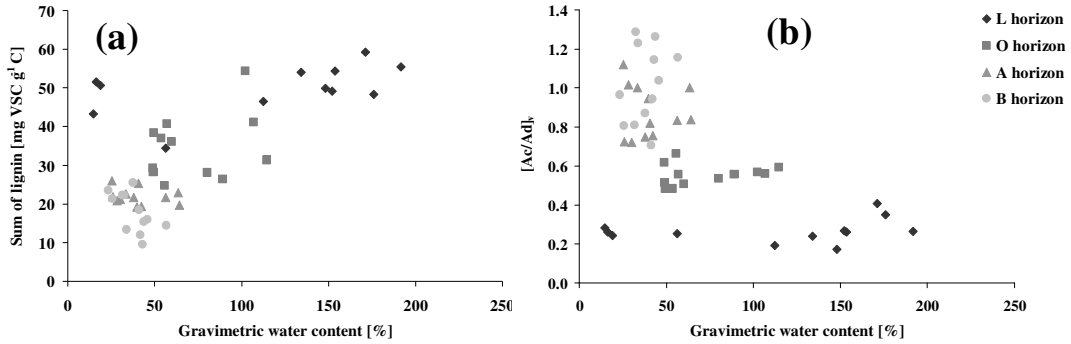

Figure 4-5: Correlation between the gravimetric water content [%] and (a) sum of VSC lignin [mg VSC g^{-1} SOC] and (b) ratio of acid to aldehyde units of vanillyl monomers ($[Ac/Ad]_v$) without control 06/2007 and 08/2007.

3.4 Plant and microbial sugars

The sum of plant sugars (arabinose + xylose) contribution to SOC decreased in the order L horizon > O layer > A horizon > B horizon (Figure 4-6a), which is in line with literature data (Rumpel et al., 2002). The sum of total sugars contribution to SOC ranged between 21 to 64 mg g^{-1} C in the organic layer and 11 to 42 mg g^{-1} C in the mineral horizon, being lower than in other forest soils (Glaser et al., 2000; Spielvogel et al., 2007). An explanation for this observation could be the fact that we did not analyze microbial hexoses, lowering total sugar concentrations. The ratio of plant to microbial sugars decreased in the order L horizon > O layer > A horizon > B horizon (Figure 4-6c), due to progressive SOM decomposition with increasing soil depth.

In the O and A horizons, microbial sugars contribution to SOC significantly decreased during the TE experiment 2007 (Figure 4-6b). The contribution of plant sugars to TOC significantly correlated with the gravimetric water content (Figure 4-7a). According to Zwiazek (1991), a decreased osmotic potential in the cell wall of *Picea glauca* needles caused a substantial increase of hemicelluloses and a decrease in pectin concentration and the other way around by drought stress. Tan et al. (1992) found that soluble carbohydrates are the major osmo-regulating solute in conifers which increased by drought stress. Buljovcic and Engels (2001) found that total soluble sugar content increased with increasing soil drought in

maize roots, too. However, in our investigated litter and soil horizons, the sum of plant sugars related to SOC decreased with drought. One possible explanation for this discrepancy could be the fact that plants produce higher sugar amounts due to osmo-regulating demands and that these sugars are not released into the soils via root exudation.

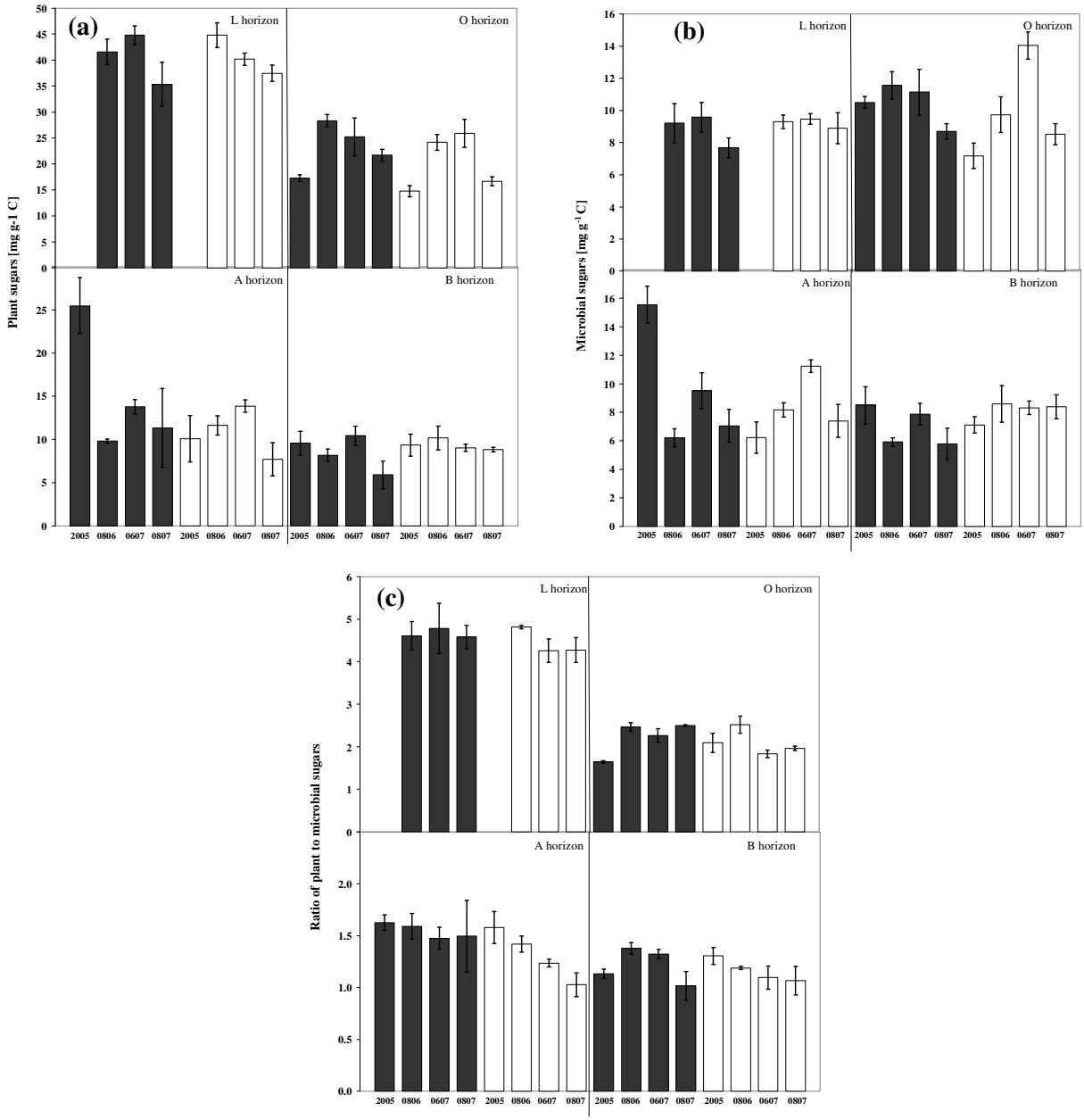

Figure 4-6: Mean value (± SE) of (a) plant sugars [mg g^{-1} SOC], (b) microbial sugars [mg g^{-1} SOC] and (c) ratio of plant to microbial sugars before (05/2005) and after (08/2006) the drying-re-wetting experiment 2006, respectively before (06/2007) and after (08/2007) the drying experiment 2007 at control (black bars) and TE plots (white bars).

However, our former laboratory experiments with exclusion of plants and thus root

exudation also showed lower plant sugar concentrations upon freezing or drying, respectively (Schmitt et al., 2008, 2010). Therefore, physical changes of soil structure and/or probably organo-mineral stabilization of sugars are a more probable explanation for the drought-induced lower sugar concentrations in soil. Alternatively, physical-chemical changes of soil structure can theoretically change the sugar extractability from soils and/or chemical changes of sugars structure can probably affect the derivatization and thus sugar quantification. Therefore, chemical alteration and stabilization could be responsible for sugar decrease with dryness.

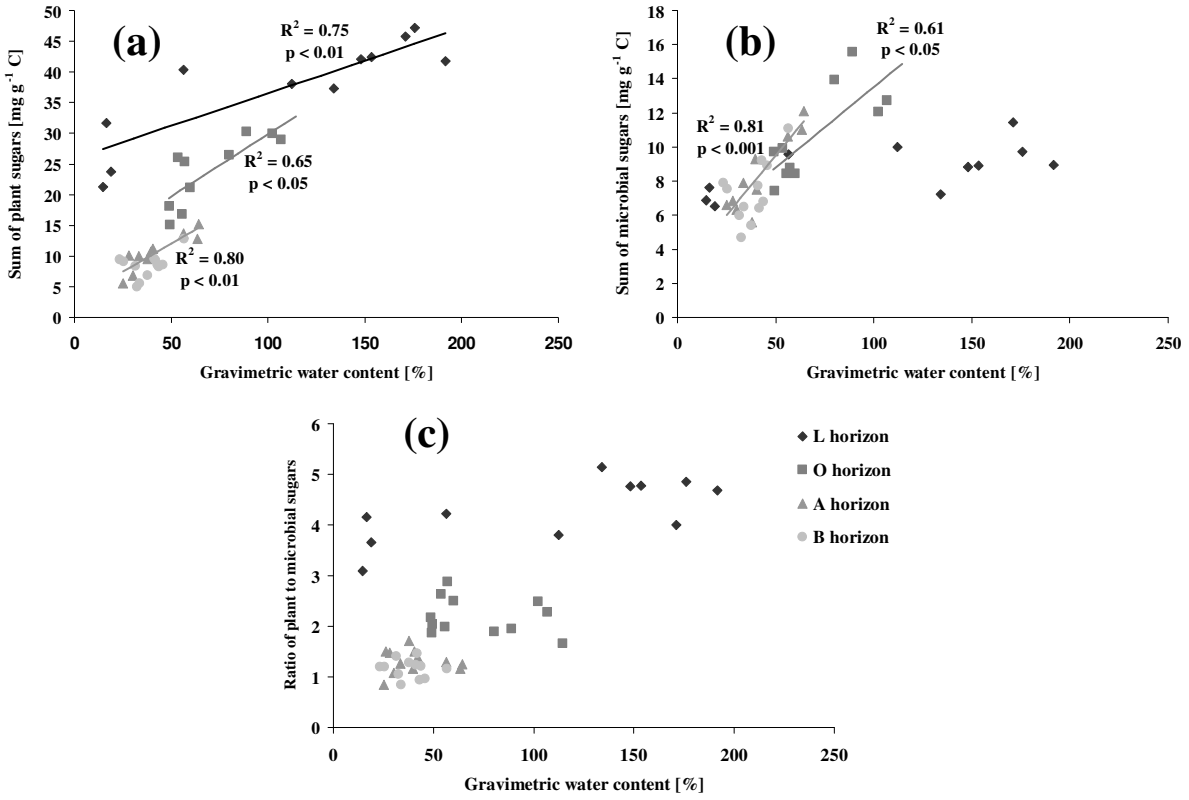

Figure 4-7: Correlation between the gravimetric water content [%] and (a) sum of plant sugars [mg g^{-1} SOC], (b) sum of microbial sugars [mg g^{-1} SOC] and (c) ratio of plant to microbial sugars without control 06/2007 and 08/2007.

The contribution of microbial sugars to SOC was significantly decreased during the TE experiment 2007 (Figure 4-6b). Also Figure 4-7b clearly demonstrated a significant correlation of microbial sugars with water content, at least in the O and A horizons. Muhr and

Borken (2009) reported that during the drying phase, smaller CO_2 fluxes were measured at the TE plots than at the control. Therefore, the microbial activity and consequently the production of microbial sugars in the soil were reduced during drought.

The ratio of plant to microbial sugars was obviously not affected by drought (Figure 4-6c), which was corroborated by the correlation with gravimetric water content (Figure 4-7c; L horizon $R^2 = 0.48$, O horizon $R^2 = 0.06$, A horizon $R^2 = 0.00$ and B horizon $R^2 = 0.01$).

3.5 Phospho lipid fatty acids (PLFA)

The sum of PLFA concentration (Figure 4-8a) increased 2006 in the order B horizon < A horizon < L horizon < O horizon, 2007 in the order B horizon < O horizon < A horizon << L horizon. In August 2007, on the O horizon of the TE plots had higher PLFA concentrations than the A horizon but the variation among replicates was also higher. The PLFA concentration ranged between 78 and 570 nmol kg^{-1} in the organic layer and between 65 and 203 nmol kg^{-1} in the mineral horizons similar to other acid temperate forest soils (Frostegard et al., 1993; Fierer et al., 2003b; Hackl et al., 2005), although different authors worked with a different number of PLFA.

In 2006, the contribution of PLFA to TOC (data not shown) increased in the order L horizon < O horizon < A and B horizons, in 2007 in the order O layer < L horizon << A horizon < B horizon. PLFA contribution to TOC in our study was comparable to data from *Pinus sylvestris*, *Picea abies* and *Betula pendula* forest soils in Finland (Priha et al., 2001). Interestingly, the same authors also found higher microbial biomass contribution to TOC in mineral soil horizons compared to organic layer as measured by both chloroform fumigation extraction and PLFA concentrations. On the other hand, soil microbial activity was higher in the organic layer compared to the mineral soil as measured by respiration indicating that soil microbial biomass is dominant in mineral soil horizons but soil microbial community being less active in mineral soil than in the organic layer.

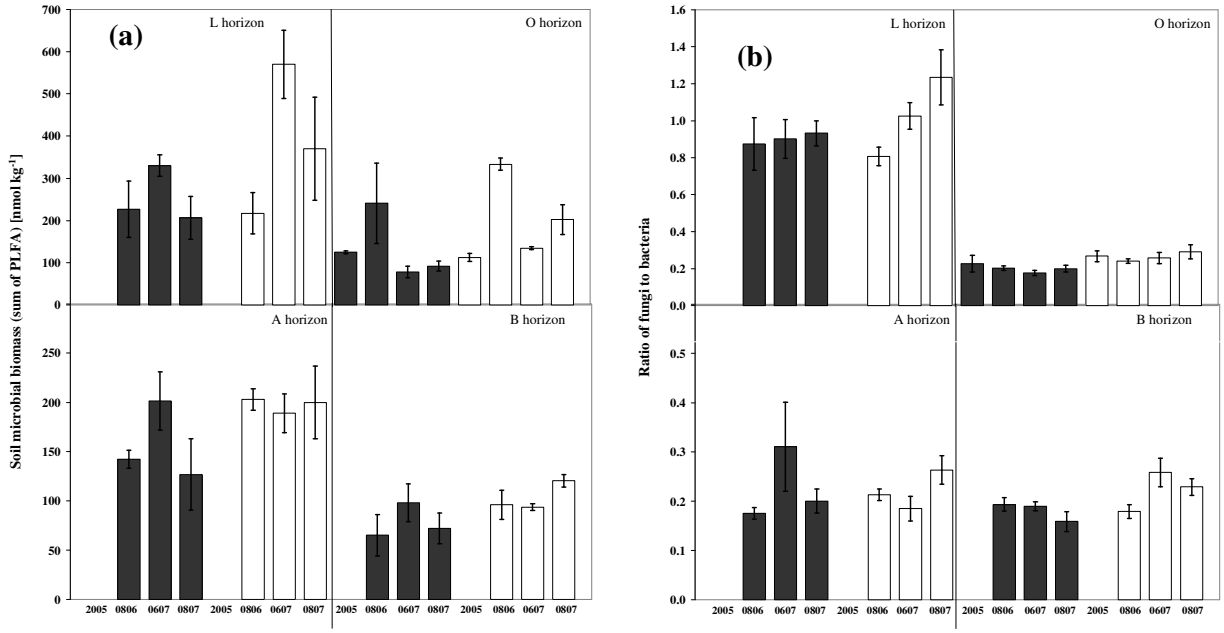

Figure 4-8: Mean value (± SE) of (a) microbial biomass (PLFA) [nmol kg^{-1}] and (b) ratio of fungi to bacteria before (05/2005) and after (08/2006) the drying-re-wetting experiment 2006, respectively before (06/2007) and after (08/2007) the drying experiment 2007 at control (black bars) and TE plots (white bars).

In 2006, soil microbial biomass increased after drought / re-wetting much stronger in the O layer of the TE plots compared to the control sites (Figure 4-8a). Soil drying and re-wetting events produced a significant stress on the soil microbial community (Fierer and Schimel, 2002) and a rapid change in soil water potential associated with re-wetting may cause microbes to undergo osmotic shock. After re-wetting, the survived microorganisms can multiply rapidly, the length of fungal hyphae and bacterial biomass increase (Jager and Bruins, 1974) due to increased nutrient availability derived from the biomass C from the death of a portion of soil biota (Jenkinson and Powlson, 1976) and from C occluded in aggregates (Vangestel et al., 1992). Even if the microbial biomass increased rapidly after re-wetting, the microbial activity was not significantly higher in our summer experiment (Muhr and Borken, 2009).

However, total PLFA concentration (Figure 4-9) did not correlate with the gravimetric water content (L horizon $R^2 = 0.10$, O horizon $R^2 = 0.09$, A horizon $R^2 = 0.04$ and B horizon $R^2 = 0.08$). Therefore, microbial biomass was probably not affected alone by changing soil

moisture regime, which is in line with literature data (Schmitt et al., 2010). Wilkinson and Anderson (2001) also found no significant changes in total microbial PLFA concentrations in a laboratory experiment (10 times remoistening after 3-to-4 days drying). It is possible that microorganisms which survive drying will multiply rapidly due to the increased nutrient availability after re-wetting (Fierer et al., 2003a) and therefore counteract any correlation with the water content.

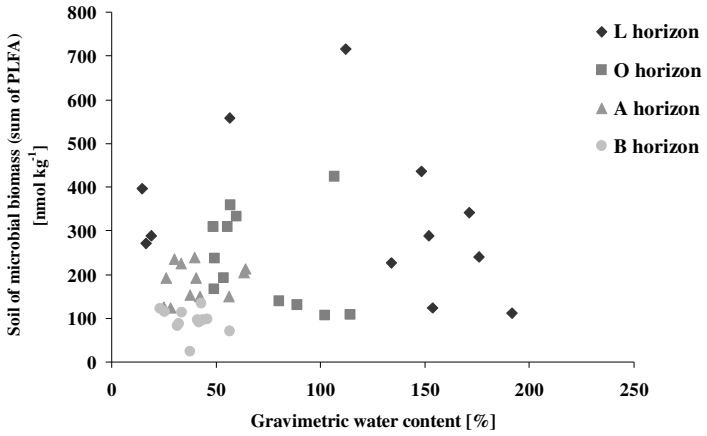

Figure 4-9: Correlation between the gravimetric water content [%] and sum of microbial biomass [nmol PLFA kg^{-1}].

However, regarding the relative contribution of individual PLFA to the sum of PLFA, some significant correlations with the gravimetric water content could be identified (Table 4-2). The saturated PLFA 16:0 ($p < 0.05$), the gram-positive markers i15:0 ($p < 0.001$), i16:0 ($p < 0.01$) and i17:0 ($p < 0.05$) and the actinomycetes markers 10Me16:0, 10Me17:0 and 10Me18:0 significantly ($p < 0.05$) increased with increasing water content in the L horizon. In the organic layer, the saturated PLFA 15:0 ($p < 0.001$) and 18:0 ($p < 0.05$), the gram-positive marker i16:0 ($p < 0.05$), and the actinomycetes marker 10Me16:0 ($p < 0.01$) significantly increased with the gravimetric water content. In the A horizon, no correlations were found and in the B horizon only the actinomycetes marker 10Me18:0 significantly ($p < 0.05$) increased with increasing water content. The fungal markers 18:2ω6,9 and 18:1ω9c ($p < 0.001$) and the Protozoan marker 20:4ω6c ($p < 0.05$) significantly decreased with increasing water content in the L horizon. In the organic layer, the gram-negative markers cy17:0 and

cy19:0 significantly ($p < 0.05$) decreased with increasing water content. In the A horizon, no correlations were found and in the B horizon only the gram-negative marker cy19:0 significantly ($p < 0.05$) decreased with increasing soil water content. Variation in the soil biophysical environment resulted in the differentiation of spatially defined bacterial communities being tolerant to moisture stress. Rapid changes in soil water potential may also select bacteria and fungi which have thicker, more rigid cell walls and compatible solutes that enhance osmo-regulatory capabilities (Schimel et al., 1999).

Table 4-2: Correlation coefficient (R^2) between the relative individual PLFA contribution to the sum of PLFA and gravimetric water content [%] without controls 06/2007 and 08/2007.

R^2 (n=12)	L horizon	O horizon	A horizon	B horizon
15:0	0.52	0.80***	0.05	0.21
16:0	0.56*	0.00	0.06	0.48
18:0	0.51	0.66*	0.12	0.14
i15:0	0.86***	0.20	0.03	0.04
i16:0	0.70**	0.66*	0.22	0.19
i17:0	0.65*	0.08	0.02	0.36
10Me16:0	0.68*	0.73**	0.03	0.02
10Me17:0	0.63*	0.00	0.07	0.15
10Me18:0	0.63*	0.15	0.28	0.62*
cy17:0	±0.00	-0.66*	±0.06	-0.46
cy19:0	-0.29	-0.59*	±0.06	-0.59*
18:2ω6,9	-0.89***	-0.41	±0.07	±0.01
18:1ω9c	-0.84***	-0.48	±0.07	±0.01
20:4ω6	-0.64*	±0.00	±0.07	-0.08
14:0	0.28	0.12	0.06	0.55
17:0	±0.00	±0.00	0.18	0.36
a15:0	0.34	0.07	0.07	0.11
a17:0	0.09	0.03	0.02	0.01
16:1ω7c	0.01	0.06	0.33	±0.00
18:1ω7c	0.08	0.01	0.27	0.06
16:1ω5c	±0.17	±0.04	-0.33	±0.06

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Apparently, drying and re-wetting affected soil microbial community structure. Gram+

bacteria and actinomycetes were reduced during water stress, while gram- bacteria, fungi and protozoa were stimulated during the drying period. These results could explain the fact that the soil microbial biomass was not affected by the moisture regime but individual microbial groups. In the deeper layers, where the quantity and quality of C substrates decline and soil moisture and temperature become less variable, soil microbial biomass and community composition were hardly influenced.

The ratio of fungi to bacteria (Figure 4-8b) decreased in the order L horizon >> O horizon ≈ A ≈ B horizons, which was in line with literature data (Fierer et al., 2003b). The ratio of fungi to bacteria significantly decreased with increasing water content only in the L horizon (Figure 4-10a). The structure of bacterial communities associated with decomposing conifer litter is highly sensitive to changes in environmental conditions (Wilkinson et al., 2002). The authors reported a dominance of fungi with increasing warmer and drier climate, while at a cooler and moister climate, bacteria dominated.

A further indicator of physiological or nutritional stress in bacterial communities is the ratio of the sum of cyclopropyl PLFA to the sum of their monoenoic precursors (cy17:0 + cy 19:0) / (16:1w7c+18:1w7c; abbreviated as cy/pre; (Kieft et al., 1997). An increase of the cy/pre ratio (Figure 4-10b) in the O layer and A horizon between June 2007 and August 2007 at the TE plots suggest that the microbes suffered from water stress. This also confirmed the correlation between the cy/pre ratio and the gravimetric water content (Figure 4-10c). In the B horizon soil moisture and temperature become less variable, microbes being more affected by carbon limitation than by climatic parameters (Fierer et al., 2003b). Microbes in the L horizon may adapt to stress they experience regularly (Wilkinson and Anderson, 2001). These results agree with the contribution of microbial sugars to SOC, which also decreased with decreasing water content in the O and A horizons.

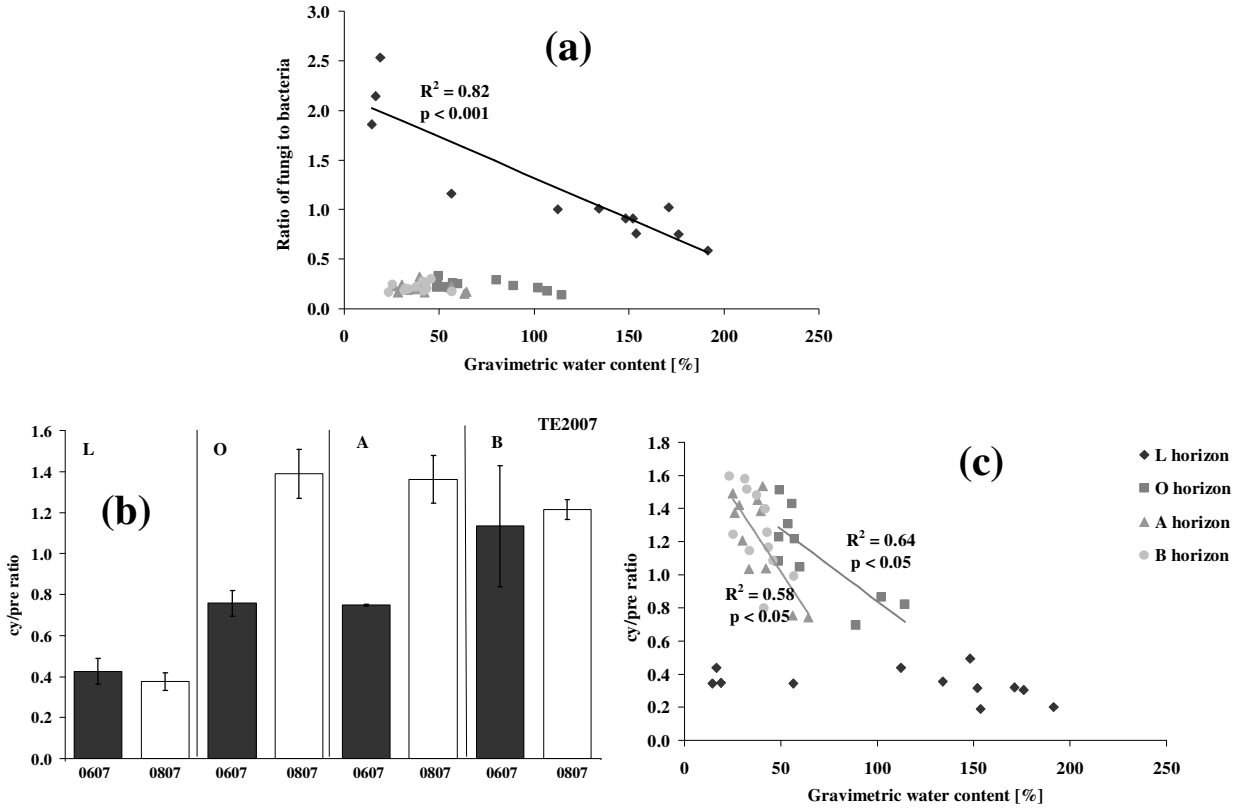

Figure 4-10: Correlation between the gravimetric water content [%] and (a) ratio of fungi to bacteria without control 06/2007 and 08/2007, (b) changes of the cy/pre ratio [(cy17:0+cy19:0)/(16:1w7c+18:1w7c)] between 12th of June (06/2007) and 13th of August 2007 (08/2007) at the control (black bars) and TE plots (white bars) and (c) correlation between the gravimetric water content [%] and cy/pre ratio without control 06/2007 and 08/2007.

4 Conclusions

Repeated moderate drought followed by intensive re-wetting in a Norway spruce forest did not influence TOC stocks and stable SOM pools (lignin). However, labile SOM pools (plant- and microbial-derived sugars) significantly decreased and soil microbial community changed. Gram+ bacteria and actinomycetes were reduced during water stress, while gram- bacteria, fungi and protozoa were stimulated during the drying period. Also the dominance of fungi increased with a warmer and drier climate and the dominance of bacteria increased with a cooler and moister climate. Increased water stress of the soil microbial community was also indicted by increasing cy/pre ratio [(cy17:0 + cy 19:0)/(16:1w7c+18:1w7c)]. However, further studies using isotope labeling approaches are required to identify underlying processes of sugar and soil microbial community dynamics in soils under changing climate. In addition,

natural variation in forest soils under field conditions is rather high. Therefore, number of replicates should be increased in the future.

Acknowledgements

This study was funded by the German Research Foundation within the Research Group "Soil processes under extreme climatic conditions" (BO 1741/3-2). Egbert Matzner and Werner Borken are acknowledged for discussion and improvement of the manuscript.

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Acknowledgements / Dank

Ich danke allen, die mir während meiner Doktorzeit zur Seite standen ganz herzlich. Mein spezieller Dank gilt:

Herrn Prof. Dr. Bruno Glaser, der mir als Doktorvater die Möglichkeit gab, an der Universität Bayreuth zu promovieren. Seine Betreuung lies mir stets den nötigen Freiraum und sorgte zugleich für motivierenden Ansporn.

Herrn Prof. Dr. Bernd Huwe, Herrn Prof. Dr. Gerhard Gebauer, Herrn Dr. Werner Borken und Herrn Prof. Dr. Egbert Matzner. Sie leisteten wichtige organisatorische Hilfestellungen und die Arbeiten an ihren Lehrstühlen bzw. ihren Abteilungen trugen wesentlich zur vorliegenden Dissertation bei.

Herrn Prof. Dr. Bruno Glaser, Herrn Dr. Werner Borken und Herr Prof. Dr. Egbert Matzner für die fachlichen Diskussionen und Korrekturlesen der Manuskripte.

Den Mitarbeitern und Helfern aus der Forschergruppe insbesondere Frau Kerstin Schulze, Frau Dr. Stefanie Goldberg, Herrn Dr. Jan Muhr, Herrn Uwe Hell, Herrn Andreas Kolb, Frau Irina Glaser, Herrn Gerhard Müller, Herrn Gerhard Küfner, Frau Iris Schmiedinger, Frau Dr. Christina Bogner und Herrn Dr. Dirk Gaul ohne deren Hilfe die Versuchsdurchführungen im Labor und Freiland nicht möglich gewesen wäre.

Die Mitarbeiter/-innen des ehemaligen Lehrstuhls der Bodenkunde, namentlich zu nennen sind hier v.a. Frau Tanja Gonter und Frau Gabriele Wittke und Herrn em. Prof. Dr. Wolfgang Zech. Sie halfen mir mit manchem gutem Rat über einige Schwierigkeiten hinweg.

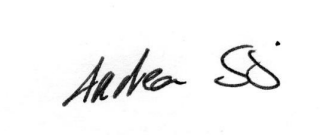
Die Studenten/-innen der „Bodenkunde-Familie“, namentlich Dr. Michael Zech, Dr. Leopold Sauheitl, Björn Buggle, Michaela Dippold, Katharina Leiber, Jago Birk, Ines Röhringer und Dr. Thorsten Ullrich, begleiteten mich auf Höhen und Tiefen und ihnen sei herzlich gedankt für die fachliche wie nichtfachliche Unterstützung.

Meinen Geschwister und meinen Eltern, sie boten mir Rückhalt in guten wie in schlechten Zeiten und sie unterstützten mich in meiner beruflichen wie persönlichen Weiterentwicklung wo und wann immer sie konnten.

Meinen langjährigen Freunden und Wegbegleitern, die mein Jammern und Klagen stets mit Geduld ertrugen und Trost spendeten.

Declaration / Erklärung

Hiermit erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ich erkläre ferner, dass ich an keiner anderen Hochschule als der Universität Bayreuth ein Promotionsverfahren begonnen habe.



Bayreuth, Juni 2011